UTMJ

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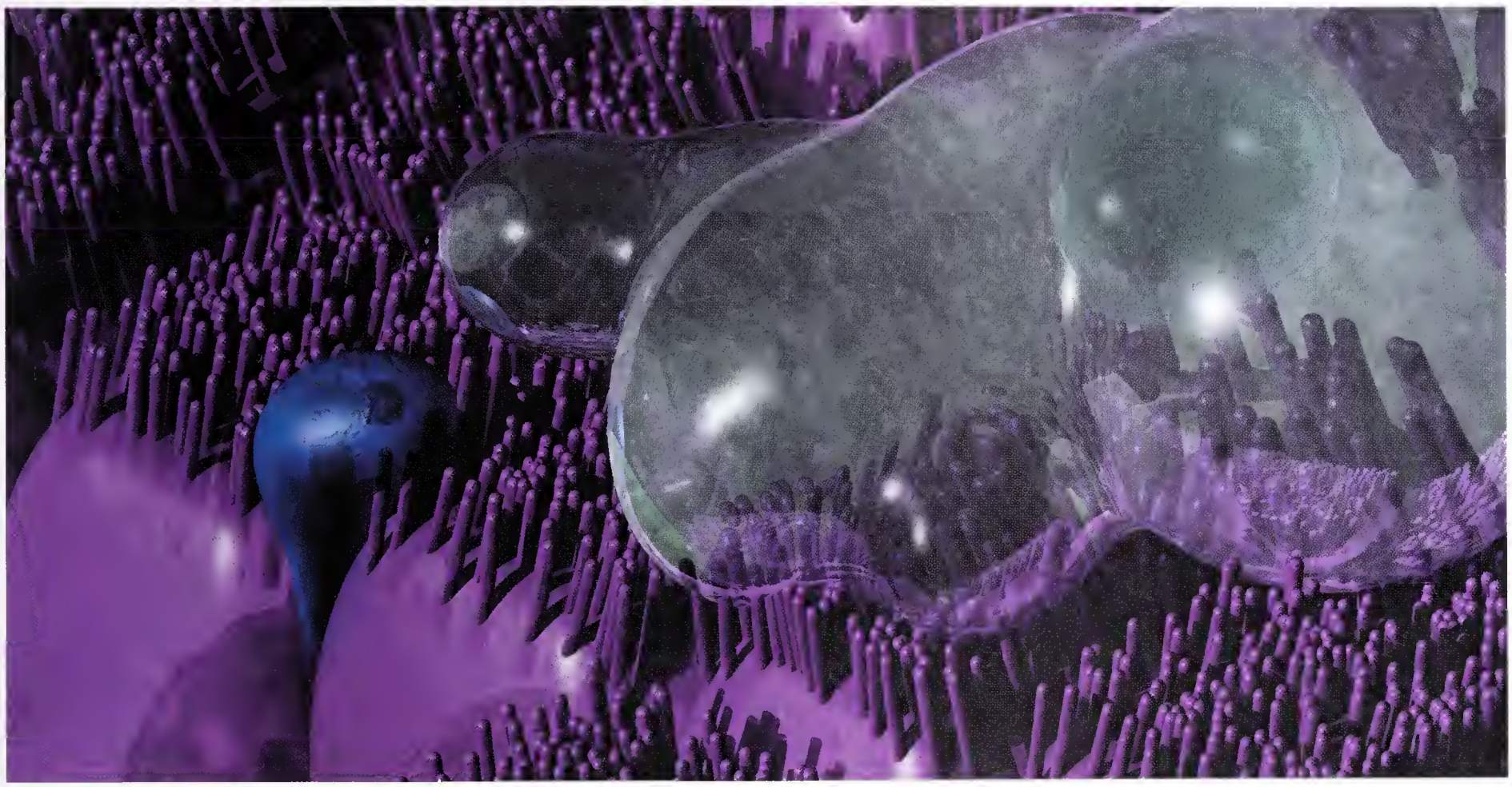

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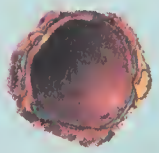

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Preface from the Editors

This issue of the *UTMJ* showcases an impressive array of articles in four colour sections. Barankin and Gottschalk have put together a useful overview of the most commonly encountered dermatological conditions seen in the community. From bench to bedside, Yang et al. report on how state of the art imaging techniques can help neurosurgeons remove malignant gliomas. Pillai and Kain provide a thoughtful and in-depth analysis of recent developments in the study of amebiasis. The final review article by Dela Cruz probes the issues and complexities of developing an HIV vaccine. These articles promise to provide something for everyone. From clinical diagnosis to fluorescent imaging, this issue will appeal to students and clinicians from diverse backgrounds.

A candid discussion with research prodigy Dr. Josef Penninger is the subject of Prolific Scientist Profiles. News and Views uncovers the creative genius of this young scientist who is a regular contributor to prestigious journals such as *Science and Nature*. Law and Ethics in Medicine concludes with questions from readers that are answered by health law expert Dr. Bernard Dickens.

For the aspiring clinician, a primer on risk assessment concludes our Evidence-Based Medicine Series. The third instalment of Back to Basics outlines a thorough approach to the blood count and film. Clinicopathological Correlation presents an exceptionally rare case that even the veteran pathologist will not find in his/her slide box. Quick Diagnosis takes a trip around the world to bring us cases from Bolivia and beyond. The focus of Morning Report is rather pungent in nature. Technology Reviews gives us a glimpse of the future of imaging in medicine and Book Reviews, as always, helps the clinician smartly expand his/her library.

With this final issue of the 77th volume, our tenure as editors has come to an end. From case reports and review articles to original articles and commentary, we have strived to offer a combination of clinical medicine and basic science that appeals to the student, educator, clinician, and scientist. We could not have reached this point without the truly superb work of our entire team. We are indebted to everyone's hard work and professionalism in meeting deadlines, reviewing submissions, and upholding the standards of the Journal.

The future of the *UTMJ* brings much promise. With the increased number of subscriptions and faculty support, the *Journal* continues to offer students a vehicle in which they can present their thoughts and ideas. In keeping with the times, our website, www.utmj.org, provides a solid foundation for growth in the expanding field of electronic biomedical journals. These achievements will ensure that our successors will maintain the high level of excellence that the *UTMJ* has delivered in the 77th volume.

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Assessing and Managing Common Skin Problems in the Primary Care Setting

Benjamin Barankin, B.A.* and Ronald Gottschalk, M.D., F.R.C.P.(C) **

Abstract

Appointments with primary care physicians for skin problems are common. It is therefore important that primary care providers be comfortable in assessing and managing a number of these problems. The more esoteric and difficult to manage cutaneous presentations are best referred to a dermatologist. However, a number of common skin problems that present in the primary care practice can be handled effectively with the proper training. This paper aimed to determine the current, common skin problems seen in the primary care setting by sending out e-mails to a number of London, Ontario family doctors to ascertain what they deemed to be most common. Using many of the responses, an approach to skin problems is presented with pertinent background, assessment, and management discussed.

Introduction

Diseases of the skin, hair and nails are a common reason for visits to family physicians and other primary care providers. Unfortunately, and for various reasons, most physicians have limited training in dermatology throughout their medical school and residency years, and thus it is no surprise that primary care physicians are less proficient than dermatologists in diagnosing accurately many skin disorders.[1–3] Considering that approximately 6% of outpatient visits relate to skin problems and that non-dermatologists manage 60% of these patients, it is important for primary care providers to be comfortable in the management of the more common cutaneous conditions.[1]

Skin Problems in General Practice

The most common skin problems diagnosed by family physicians in one study included (in descending order): dermatitis, pyoderma, warts, tinea infections, epidermoid cysts, candida, acne and actinic keratoses; the same study found that the top 10 and top 20 most common diagnoses accounted for 65% and 82% respectively, of all skin-related diagnoses.[2] Primary care providers should be able to recognize and manage the twenty most common skin problems. As well, they need to recognize less common but serious skin conditions (e.g. melanoma, pemphigus), and manage or refer them appropriately.

Primary care providers should be able to both diagnose and manage skin problems effectively. One technique, essential to the management of many common skin lesions, is liquid nitrogen cryotherapy. Cryotherapy is a popular treatment modality because it is relatively inexpensive, easy to use, does not require local anesthesia, and has few complications.[4] It is most commonly delivered as liquid nitrogen or nitrous oxide, and the spray canister approach is the preferred delivery route. The use of cryotherapy should be preceded by an understanding of which lesions are amenable to such therapy, the duration of treatment, and the proper use of margins in therapy. It has been shown to be effective in the treatment of: actinic keratoses, seborrheic keratoses, dermatofibroma, keloids, molluscum contagiosum, condylomata and common warts, and benign nevi.[4]

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A good knowledge of the different vehicles used in topical medications (e.g. ointments, creams, lotions) is an important consideration in managing cutaneous problems. Topical corticosteroids comprise a large part of the arsenal for managing skin problems. Their side effects are a function of steroid potency as is their cost (more potent steroids are more expensive). Primary care providers should be comfortable in prescribing most low and some medium potency steroids, but should reserve more potent steroids for dermatologists.

The management of skin lesions in children differs from the approach used in adults. Physicians should avoid unnecessarily traumatizing or scarring children, and so the use of invasive diagnostic techniques like skin biopsies should be reserved for when they are essential to the management of the disease.[5] Proper education of the parent and child, the use of distraction to allay fear, and an appreciation of the maturity of the child when deciding on treatment, are all beneficial in the management of skin problems in the pediatric population.[5]

Table 1
Focused History Taking[7,11]
Mnemonic: ABCDEF PPP

Acute vs. Chronic – time of onset
Behaviour – do lesions come and go, recur in the same area
Change in size or colour
Discomfort – itching, burning/tingling, pain
Effects of external agents – sun, detergents
Family History – psoriasis, atopic dermatitis

Pills & Allergies
Past history – sun burns & exposure, similar rash
Previous Treatments – steroids, moisturizers, ointments/creams

Survey

An informal survey of the most common skin problems noted by family doctors was conducted in the London, Ontario region. Of 15 family physicians surveyed by e-mail, 9 responded. Based on these findings, the diagnosis and current management of some of the most common skin conditions observed in the family practice setting is presented.

Approach

The first step in dealing with a patient presenting with a skin problem is the visual examination of the lesion. An appropriate history (Table 1) then augments the exam, helps to establish the differential diagnosis and previous responses to treatment. As well, being able to describe lesions in an organized manner (Table 2) and using the proper terminology (Table 3) will improve clinical diagnosis acumen, management and necessary referrals.

Acne Vulgaris

This is an inflammation or blockage of pilosebaceous units, most commonly on the face, but also affecting the back and trunk.[6,7] It is usually more severe in males, and has a lower incidence in Asians and blacks.[6] Although it most commonly affects adolescents (age 10-17 in females, 14-19 in males; 100% of boys and 90% of girls will have some acne), it can extend into, or even begin in, adulthood.[6] It is important to be aware of the psychological impact of acne as it can lead to low self-esteem, depression, and embarrassment (among other things).[8] The classic features are open (black) or closed (white) comedones, inflammatory papules and pustules.[7] Different manifestations include comedonal acne, papulopustular acne, nodulocystic acne, and acne conglobata.[6] Pitted, depressed or hypertrophic scarring may occur with severe acne (especially nodulocystic).[6] Steroids and oral contraceptives are common medications that may exacerbate the condition. As well, emotional stress, endocrine fac-

Table 2
Approach to Describing Skin Lesions[7,11]
Mnemonic: LES T. CABS

Order of Description		Descriptives
Location & Distribution		Symmetrical, Asymmetrical, Sun-Exposed Flexural/Extensor, Acral (hands/feet)
Erythema?		Erythematous or Non-Erythematous
Surface Features	***soft palpation*	Normal/Smooth, Scaly, Warty, etc.
Type	***deep palpation*	Flat or Raised, Consistency, 1° or 2°
Color		Pink/Red/Purple, White, Brown, Yellow
Arrangement		Single or Multiple, Discrete, Unilateral, Generalized, Disseminated, Grouped, Annular, Linear, Serpiginous
Border & Shape		Well or Poorly Defined, Active Edge, Round/Oval/Irregular/Pedunculated
Special Sites		Scalp, Mouth, Nails, Genitalia

Table 3
Common lexicon in dermatology[6,7,11,14]

Description	Definition
Annular	Arranged in a ring shape
Atrophy	Depressed surface due to thinned epidermis or dermis
Bulla	Blister > 1 cm
Burrow (= papule)	Linear 'S' shaped papule 3-5 mm, found in scabies
Comedone (= papule)	Plugged (pilo) sebaceous follicle *1. Closed or whitehead* *2. Open or blackhead*
Crust (or scab)	Dried serum, pus, or blood
Cyst (= papule/nodule)	Epithelium-lined cavity containing fluid, pus or keratin
Ecchymosis ("bruise")	Large confluent area of purpura
Erosion	Partial loss of epidermis. *** Heals without scarring*
Erythema	Redness that blanches on pressure
Excoriation	Local damage due to scratching
Exudate	Serum, blood or pus accumulated on skin surface
Fissure	Linear split in epidermis or dermis.
Keratin/Horn	Rough, uneven surface. Difficult to pick off, unlike crust.
Lichenified	Thickened epidermis (& increased skin markings) due to excess scratching
Macule	Flat, pigmented lesion < 1 cm
Nodule	Raised lesion > 1 cm with rounded surface *** usually dermal pathology (no surface)*
Papule	Raised lesion < 1cm
Patch	Flat, pigmented lesion > 1 cm
Petechia	Small, nonblanching, red-brown macules
Plaque	Raised lesion > 1 cm (diameter>>thickness) *** usually epidermal pathology [surface (e.g. scaling, crust etc.)]*
Purpura	Red or purple skin which doesn't fade with pressure
Pustule	Pus-filled lesion < 1 cm
Scaly	Dry/flaky surface
Scar (cicatrix) (= macule/papule/plaque)	Healed dermal lesion 2° to trauma, surgery, etc.
Serpiginous	Snake-like in appearance
Telangiectasia	Small dilated blood vessels
Ulcer	Full-thickness loss of epidermis & some dermis. There will be either exudates or crusting present. *** heals with scarring*
Umbilicated	Round depression in center of surface
Vesicle	Blister < 1 cm
Warty/Papillomatous	Finger-like/round projections on a surface
Wheal (= papule/plaque)	Transient swelling due to dermal edema (synonymous with urticaria)

tors, occlusion and pressure on the skin have all been implicated in worsening the condition. Despite common folklore, neither foods (e.g. chocolate) nor poor hygiene cause acne, and sunlight does not improve acne.[6,8]

Treatment:[6,7,9-11]

Mild Acne: *Topical*

- Topical Antibiotics (clindamycin, erythromycin)
- Benzoyl peroxide (2%, 5%, or 10%) qhs or bid
- Topical retinoids (cream or gel)

* most common side effect: mild irritation (creams are less irritating than gels).

** benzoyl peroxide and topical retinoids are contraindicated in pregnancy.

Moderate Acne: *Oral + Topical*

- Oral tetracycline, minocycline, or erythromycin.
  * *Note: do not give tetracycline or minocycline to children or pregnant women.*
- In females, one may try oral estrogen dominant combined with progesterone birth control (e.g. Marvelon, Tricylin, etc.) or estrogen with anti-androgen therapy (e.g. Diane 35).

Severe Acne

• Isotretinoin (Accutane): used for severe, resistant, nodulocystic acne with scarring (typically 1 mg/kg/day). 90% of people respond to a first course, with a long-term cure of 65%. Best managed by a dermatologist.

* Isotretinoin is teratogenic, therefore proper contraception and pregnancy testing is indicated in all females. Fasting blood lipids (esp. triglycerides and cholesterol) and liver biochemistry should be assessed monthly. Dry nose, mouth, and lips are common side effects. Night blindness and mood changes have been reported occasionally as well. Sometimes a second or third course of isotretinoin may be needed.

Actinic Keratosis (also called Solar Keratosis)

These are small (<1 cm), single or multiple, discrete, sandpaper-rough, scaly lesions.[6,10] They occur on chronic sun-exposed and damaged skin (most common in the elderly).[6] They are found primarily on the head and neck, as well as the dorsum of the hands and forearms.[10] These lesions are considered pre-cancerous, with 1 in 1,000 lesions developing into squamous-cell carcinoma if left untreated.[6]

Treatment:

Most commonly with liquid nitrogen cryotherapy (topical 5-fluorouracil may also be used by those with experience since it is extremely irritating).[6]

Dermatofibroma (also called Fibrous Histiocytoma)

A very common, small (<1 cm), firm, raised nodule, which is slow-growing (neoplasm) and usually painless (Figure 1).[10,12] A skin colored/pink/red/brown nodule (with occasional brown circumference) that is usually solitary, more common in females and the middle aged, and usually found on the extremities (especially legs).[6,10,11] They are sometimes associated with a recent history of trauma, such as an insect bite or ruptured cyst.[10,12] Clinical diagnosis can be support-

ed by the presence of a 'Dimple sign' (lateral compression with finger and thumb produces a depression/dimple).[6]

Treatment:[6]
Unnecessary due to benign nature of lesion, but liquid nitrogen cryotherapy can be effective. Surgical excision is also an option.

Figure 1. Dermatofibroma
Solitary, pink, raised, 2.5 cm nodule on the dorsal forearm. Figure courtesy of Dept. of Dermatology - University of Iowa College of Medicine, http://tray.dermatology.uiowa.edu/DermImag.htm

Hand, Foot, and Mouth Disease (HFMD)
A self-limited (7-10 days) systemic infection usually caused by a coxsackie viral infection.[6] Most commonly seen in young children, it is highly contagious and spreads by direct contact.[6,8] A clinical diagnosis, it appears as multiple small grey vesicles with a red halo on the fingers and toes, and ulcerative oral lesions (Figure 2).[6,8,11] Often there is a low-grade fever, malaise and complaints of a sore mouth, although symptoms may be more severe in some.[6]

Treatment:[6]
Symptomatic

Figure 2. Hand, Foot and Mouth Disease
Multiple grey vesicles with a red halo on the palmar hand. Figure courtesy of Dept. of Dermatology - University of Iowa College of Medicine, http://tray.dermatology.uiowa.edu/DermImag.htm

Impetigo
An infectious skin disease, spread by direct contact, and most common in children. It can be 1° or 2°, and there are three types: nonbullous impetigo, bullous impetigo, and ecthyma. Most cases (>90%) are due to *Staphylococcus aureus*, although occasionally may be due to *Group A Beta-hemolytic Streptococcus* (GABHS), or a combination of both.[6,7] It presents as a weeping area of 1-3 cm rounded lesions which heal with honey-coloured crusts (Figure 3).[6] Lesions typically last days to weeks and resolve promptly with proper treatment.

Treatment:[6]
Preventive (check family members for impetigo), topical (mupirocin, fucidin, or cantharidin), or systemic (depends on organism, although erythromycin or cephalexin are good options for 10 days).

Figure 3. Impetigo
Multiple honey-coloured crusts on the arm in the setting of atopic dermatitis. Figure courtesy of Dept. of Dermatology - University of Iowa College of Medicine, http://tray.dermatology.uiowa.edu/DermImag.htm

Intertrigo
A malodorous dermatitis of flexural regions often secondary to bacterial or yeast infections and most commonly affecting the axillae, inframammary, groin, and intergluteal areas.[6,9] Initially appears as pustules on an erythematous base which become confluent.[6] Should be differentiated from erythrasma and psoriasis inversus. Management involves keeping the area clean, cool, dry, and separated.[9]

Treatment:[6,9]
Options include nystatin or imidazole creams bid, as well as topical mild steroids and powders.

Lice (Head)
This is an infestation of the scalp by wingless insects spread by direct contact (shared items or head-to-head), occurring mainly in children.[6,11] Infestation is not related to poor hygiene. Head lice are difficult to see, particularly as most patients have < 10 lice and so the diagnosis is by clinical findings confirmed by finding nits or lice.[6] Usually asymptomatic, itching is in some cases intolerable and can result in secondary infection (note that occipital or cervical lymphadenopathy may be suggestive of lice).[6,11] The eggs/nits are 1 mm, oval, grey-white, and firmly attached to the base of the hair. These parameters form the clinical basis for diagnosis and differentiation from dandruff where the scales are easily removed.[6,11]

Prevention:

Avoid contact with possible contaminated items (e.g. hats, combs). Bedding, clothing, and head gear should be washed and heat-dried, and the environment vacuumed (lice can survive up to 55 hrs off the head).[6] Contacts should also be treated.

Treatment:[6,13]

1. permethrin 1% (can use 5% if it fails): reduced reinfestations due to protective residual, but should repeat treatment in 7 days. Preferred treatment.
2. lindane: 90% success rate, but potential side effects due to high absorption†.
3. other: pyrethrin, malathion, petrolatum (rubbed into scalp to suffocate lice)

* Treat entire body for all of the above.

** Most schools will not allow students to return until nits are gone, and so nit removal with a fine comb (nit comb) may be used.

*** Note that resistance to all topical agents has been reported.

† *Serious sequelae such as seizures and aplastic anemia have been associated with the use of lindane; it should not be used in pregnant or lactating women, very young children, and those with extensive dermatitis. Bathing prior to application should be avoided.*

Molluscum Contagiosum

A self-limiting (lasting between 6-9 months) viral infection of the skin caused by the pox virus, most commonly affecting children (but also sexually active adults).[6,10,11] These are 1-5 mm smooth, firm, white/pink umbilicated papules found anywhere on the skin (Figure 4); they may be single or multiple (especially if immunocompromised; multiple facial mollusca may suggest HIV infection).[6,10,11] Although itching, tenderness or pain are uncommon, roughly 10% of patients develop an eczematous dermatitis around the lesion.[10]

Treatment:[6]

Includes curettage, liquid nitrogen cryotherapy (10-15 seconds), electrodessication, topical cantheradin or laser surgery. Treatment in immunocompromised host may be unnecessary.

Figure 4. Molluscum Contagiosum

Multiple, smooth, pink, umbilicated papules on the trunk. Figure courtesy of Dept of Dermatology - University of Iowa College of Medicine, http://tray.dermatology.uiowa.edu/DermImag.htm

Scabies

A parasitic infection usually transmitted by direct contact. Older patients in nursing homes are more prone to infestation, as are young children and sexually active young adults.[6] Patients present with an itchy, eczematous, papular rash, most commonly in the finger webs, flexor aspect of wrists, elbows, buttocks, and genitalia, with sparing of the head and neck (Figure 5).[6,9] With the initial infection, sensitization (and pruritus) take a few weeks to develop, but after a reinfestation, pruritus develops within 24 hours.[6] Because a clinical diagnosis can be difficult to make, a scabies infection should be in the differential in the setting of a persistent generalized pruritus.[6] Pruritus is often intense, widespread, and worse at night, such that it interferes with sleep.[6,14] Unfortunately for some patients, this condition has been misdiagnosed and mistreated as an eczema for long periods of time.[14]

Treatment:[6,13,15]

1. permethrin 5% cream (the most reliable topical scabicide, no toxicity, residual effects)
2. lindane 1% lotion/cream (apply on successive nights) or
3. other: malathion

Treatment is effective in over 90% of cases. Clothes and bedding must be laundered in hot water. Make sure to treat close personal and household contacts at the same time, often regardless of symptoms. Be aware that pruritus may persist for weeks after successful treatment.

Figure 5. Scabies

Burrow on side of finger. Figure courtesy of Dept. of Dermatology - University of Iowa College of Medicine, http://tray.dermatology.uiowa.edu/DermImag.htm

Skin Tag (also called acrochordon or cutaneous papilloma)

Skin tags are common, soft, skin or tan-colored pedunculated polyps. They occur commonly in intertriginous sites, and increase in size and number over time.[6,12] They are more common in older people, females (especially during pregnancy), and in obese patients.[6] Usually asymptomatic, they may become bothersome and tender after trauma or torsion. Skin tags are completely benign and thus only require management if the patient is symptomatic or for cosmetic purposes.

Treatment:[6,12]

Snipping with scissors, liquid nitrogen, or by electrodesiccation.

Tinea Pedis (also called athlete's foot)

A dermatophytic infection of the feet. Appears as erythema with scaling and maceration.[6] Usually asymptomatic, but may be itchy (painful if infected). Often a chronic condition made worse by hot climate. Two main varieties, the interdigital (Athlete's foot) which can be a source for cellulitis of the foot, and the hyperkeratotic or mocassin foot.

Treatment:[6,9]

1. Topical: miconazole or clotrimazole applied bid, ketoconazole OD, terbinafine cream
   *nystatin does not work because it has no systemic absorption.
2. Systemic (if extensive or topical failure): itraconazole, terbinafine

Prevention:

shower-shoes in public facilities, wash feet regularly.

Tinea Versicolor (also called Pityriasis Versicolor)

A chronic, asymptomatic scaly rash of different colours (white, orange-brown, or dark brown) with round macules usually < 1 cm which liberate scale when scratched.[6,11] Caused by an infection by the yeast *Pityrosporum ovale* (formerly classified as *Malassezia furfur*), it is more common in young adults and most commonly appears on the upper trunk.[6,11] Clinical suspicion along with a positive KOH preparation make the diagnosis.

Treatment:[6,9]

Options include selenium sulfide (2.5%) lotion or shampoo applied daily for 15 minutes for 1 week. Can also treat with oral ketoconazole or itraconazole, and terbinafine cream that are more expensive. Topical imidazoles are also useful.

Warts/Verruca

Common Warts/Verruca Vulgaris: This is a human papilloma virus infection of the epidermis that is transmitted directly through broken skin. They are found on the fingers, palm and dorsum of the hand. They appear as firm, rough, skin or brown coloured papules with tiny black dots on the surface.[11] Treatment is only necessary if the warts are bothersome or for cosmetic purposes. In fact, most warts disappear spontaneously after an average of 2 years.[11]

Treatment:

The mainstay is cryotherapy, which may need to be repeated on several occasions. Daily treatment with over-the-counter wart removal preparations (17% salicylic acid, 17% lactic acid) may be helpful, but produces a slower response.[9]

Plantar Warts/Verruca Plantaris: Appear as discrete round papules with a rough surface, surrounded by a layer of hyperkeratosis.[11] Although less a cosmetic issue, these are more likely to be treated because the hyperkeratosis around the wart may cause pain when walking.[11,14]

Treatment:

May consist of paring down the hyperkeratosis with a scalpel or pumice stone which may relieve the pain, use of a 40% salicylic acid plaster, or a 17% salicylic acid/17% lactic acid over-the-counter preparation.[11] If these don't work, liquid nitrogen (possibly several courses) may be used. A further treatment option involves the use of a carbon dioxide laser that can destroy the lesion, but this requires referral to an appropriate dermatology clinic.

Conclusion

The primary care physician is frequently confronted with patient concerns relating to the skin, hair and nails. Many common skin problems can be handled effectively with the proper training. A primary care provider comfortable with the diagnosis and management of common skin problems, and with an appreciation of situations requiring referral, is better able to provide the necessary medical care to their patients.

Acknowledgment

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Intraoperative Neurosurgical Fluorescence Microscopy: Preliminary Report on Intraoperative Detection of Glioblastoma Multiforme by a Multispectral Fluorescence Guidance System

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Abstract

Intraoperative identification of tumour can help to define the tumour extent and detect residual tumour tissue, which may increase the effectiveness of both conventional surgical resection and Photodynamic Therapy (PDT). In this preliminary report, we present the first clinical demonstration of a novel Multispectral Fluorescence Guidance (MFG) system that performs non-contact fluorescence spectroscopy and microscopy intraoperatively. METHODS: At twenty-four hours before surgery, patients were administered Photofrin™ i.v. at 2 mg/kg. Intraoperatively, tissue fluorescence was excited by 405 nm light and detected in the visible and near infrared wavelengths (480 ~ 720 nm). Both spectroscopy and multi-spectral imaging were made. Fluorescence measurements were made during and after the surgical resection of tumour tissue, as well as before, during, and after PDT. Biopsy samples were taken from the tumour resection region, and examined histologically and spectrofluorometrically to determine the pathology and tissue concentration of Photofrin. RESULTS: Normal brain tissues showed no elevated porphyrin fluorescence, in contrast to tumour tissue which displayed characteristic red fluorescence centered at 640 nm. After surgical resection, the MFG system was able to detect residual tumour tissue with sub-millimeter resolution intraoperatively. During PDT, the MFG system detected photobleaching of Photofrin intraoperatively. CONCLUSION: Fluorescence microscopy is feasible during neurosurgery. Our observations suggest that Photofrin used for PDT may serve as a fluorescence label for malignant gliomas. An intraoperative device such as the MFG system can utilize such label for surgical guidance, which might enhance the completeness of tumour removal and improve survival rates. Intraoperative measurement of photobleaching may also be used in improving dosimetry of PDT.

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Introduction

In vivo fluorescence measurements of tissue has the potential of providing new tools in clinical oncology, for screening and diagnosis of early-stage malignancy, for defining tumour extent and spread to adjacent tissues and as a guide for optimizing localized treatment of solid tumours.[1] Early attempts at *in vivo* laser fluoreseence bronehoseopy, developed by Profio *et al.*,[2] paved the way for developing eommercial instruments sueh as the lung imaging fluoreseenee endoseope.[3] Reeent advanees in exeitation light sources, especially blue and UV lasers, improved the quality of fluorometrie methods by providing speeifie wavelength exeitation at high power density. Other improvements in photonies teehnology, sueh as thermo-electrieally-eooled eharge eoupled deviees, fiber optieal speetroseopy, and advanees in digital imaging/proeessing, have allowed extension of *in vivo* fluoreseenee teehnique into a wider array of medieal applications, sueh as intra-operative surgieal guidance.

Due to the aggressive growth pattern and the infiltrative nature of glioblastoma multiforme, the elinieal eourse of these tumours rarely exeeeds 18 months, despite aggressive treatment using surgieal removal, radiotherapy and ehemotherapy.[4-8] One of the important determinants of prognoses for malignant gliomas is the extent of surgieal reseetion.[9-12] Guided operative resection may improve the treatment outeome in patients with these tumours.

CT and MRI offer superb image resolution of anatomieal strue-

ture preoperatively, intraoperative image reconstruction is of limited values in intrinsic brain tumour resection because of intraoperative brain shifts which can be caused by surgical retraction, resection and cerebrospinal fluid drainage.[13-16] Intraoperative real time imaging avoids the aberration of brain shift; however, these techniques are based on highly specialized imaging systems, such as MRI and PET which are only feasible in large surgical centers. Intraoperative ultrasound has been attempted,[13,17] but the requirement of coupling made this modality cumbersome for open-field surgical use.

Optical imaging modalities offer good resolution, and the advance of fluorescence molecular probes provide possibilities for imaging specific tissues with high contrast. Previous attempts of intraoperative imaging of malignant gliomas using fluorescence markers showed some success using non-imaging techniques or with modification of existing surgical microscopes.[18-23]

Previous work in our lab investigated the differential uptake of hematoporphyrin derivatives between normal and tumour brain tissue in a rabbit model.[24] Our efforts in developing non-contact tissue spectroscopy led to the current work.[25-30] In this paper, we present an intraoperative Multispectral Fluorescence Guidance (MFG) system with optics optimized for fluorescence detection. The MFG system can perform non-contact fluorescence spectroscopy and microscopy simultaneously. We also present a preliminary report of the clinical demonstration of the MFG system in detecting residual tumour during the surgical resection of glioblastoma multiforme, as part of clinical trials of Photodynamic Therapy (PDT) using Photofrin™ (hematoporphyrin derivative, QLT Phototherapeutics, Vancouver, British Columbia) to treat malignant gliomas.[31]

Apparatus

The MFG system is an optical illumination and detection platform specifically designed for open-field surgical procedures.[28-30] Mounted on a suspension arm, the MFG system illuminates the surgical field with 405 nm light at 2.8 mW/cm^2 uniformly across an area of 3 cm in diameter. This illumination wavelength matches the absorption peak of Photofrin (Figure 1). The fluorescence emitted from the surgical field is collected by a multispectral digital camera, which is an integral part of the MFG system, covering 480~720 nm at 755-484 pixels with 10-bit resolution. Images are obtained in separate wavelength bands each with 20 nm bandwidth. The working distance is 50 cm, which allows the system to be positioned sufficiently far from the surgeon's hands (Figure 2). The field of view is 3.3-2.5 cm^2 and the depth of focus is 2 cm, which are adequate for the geometry of open-field neurosurgery. Over the entire field of view, the spatial resolution of the multispectral camera is 0.15 mm (Figure 3) and the spectral resolution is 20 nm. Using tissue phantoms with optical absorption and scattering properties similar to brain tissue, the minimum concentration of Photofrin detectable by the camera was 0.05 μg/g at the tissue surface and 0.1 μg/g at 0.5 mm below the surface (Figure 4). A fiber optic spectrograph, integrated with the MFG system, performs non-contact fluorescence spectroscopy focused at a small area (diameter ~ 0.6 mm) of the surgical field. Using normal operating room lighting, the MFG system can also take white-light images of the surgical field. The total measurement acquisition time for a white-light image, a fluorescence image, and a fluorescence spectrum is less than 30 seconds.

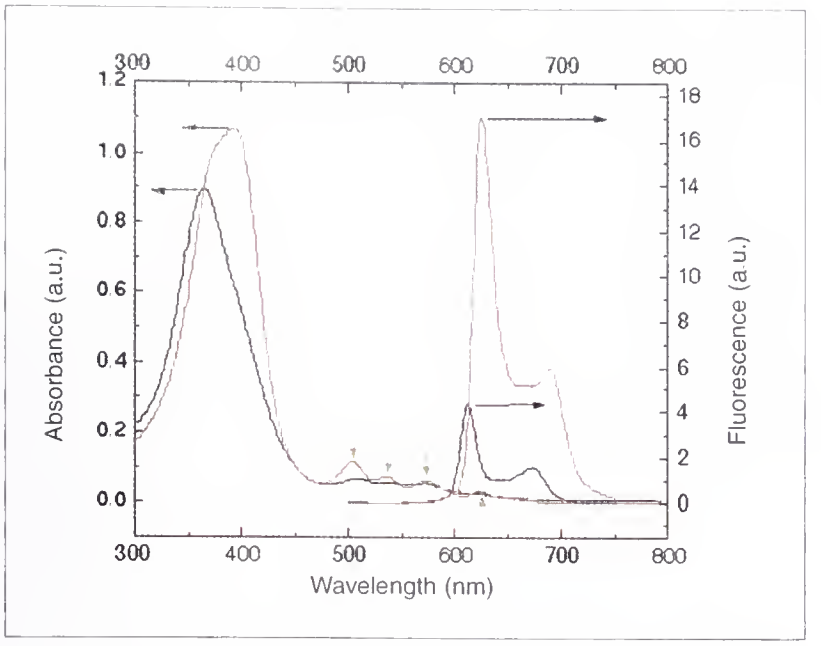

Figure 1. *Absorption and fluorescence spectra of monomerized Photofrin (red) and aggregated Photofrin (black) samples. The green arrows indicated the minor absorption peaks, which were more pronounced in the monomer sample. The fluorescence spectra were measured with the excitation fixed at 405 nm.*

Figure 2. *The MFG system during an operation.*

Methods

As part of existing phase III randomized prospective clinical trials of PDT in the treatment of supratentorial gliomas using Photofrin,[31] 4 patients (2 male, 2 female, age 40 ~ 57) were imaged by the MFG system. Two additional patients (# 1, 6) with benign cystic meningioma and recurrent cervical chordoma were also imaged (see Table 1). For patients undergoing PDT, 2 mg/kg of Photofrin was administered i.v. 24 hours before surgery. When irradiated with light at 630 nm, Photofrin is photosensitized and produces singlet oxygen and free radicals which are toxic to the surrounding tissue.[31]

The positioning of the MFG system in the operating room was similar to that of conventional operating microscopes with

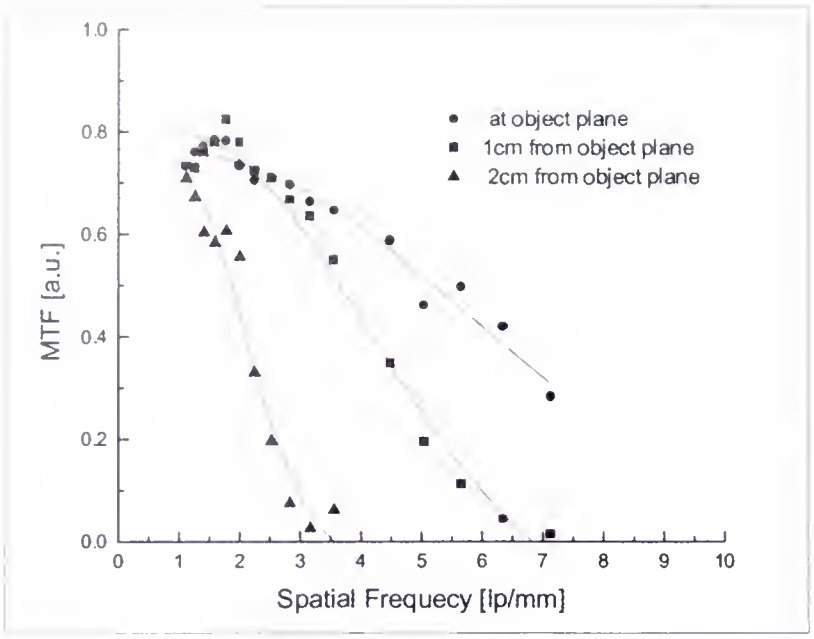

Figure 3. *Modulation Transfer Function (MTF) of the MFG system when the target was placed at different distances away from the object plane. The solid curves are Gaussian fit of the experimental data points. Within the depth of focus (+/- 1 cm), the MTF was >10% at 6.5 line pairs per mm (~ 0.15 mm spatial resolution).*

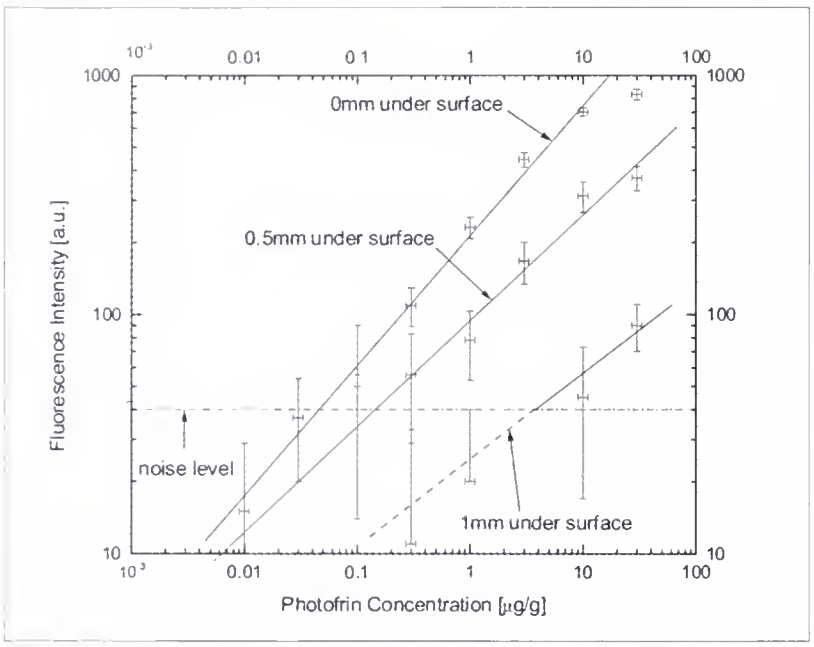

Figure 4. *Detected fluorescence intensity as a function of Photofrin concentration in an optical phantom of normal brain at different depth from the tissue surface. The noise level, indicated the detection limits of MFG were 0.05 mg/g at tissue surface and 0.1 mg/g at 0.5 mm below the surface.*

manual suspension arms. The conventional operating microscope was used when necessary for the surgical resection. After the surgical resection, the MFG was focused on to the resection plane. Prior to taking images, the surgical field was irrigated to remove residual blood,[20,21] which could significantly attenuate the fluorescence excitation light.[34] A complete MFG measurement included a white-light image, a corresponding fluorescence image, and a fluorescence spectrum. The white-light image was taken with the operating room light on. The fluorescence image and spectroscopy were obtained with the operating room light turned off for a short period (<1 minute). Minimum modification was done to the neural surgical operating room, with dark blinds installed on windows and doors to block environment lighting. During a typical operation, two complete MFG measurements were made, one after the surgical resection and one after the PDT. Biopsy was taken from the tumour resection cavity and examined histologically and spectrometrically to determine the pathology and concentration of Photofrin uptake. For all of the above patients, data obtained by the MFG system were not used to alter the surgery or PDT treatment.

The histological analysis was performed by a neuropathologist blinded to the treatment. The Photofrin uptake was measured by an unbiased technologist using a spectrofluoremeter (PTI-710, Photon Technology International, London, Ontario). For patient undergoing PDT, red laser illumination at 630 nm of the surgical cavity was performed after resection. The laser light dose given was either 40 J/cm^2 or 120 J/cm^2, depending on the randomization. Photobleaching was examined in one PDT patient (# 3), when three complete MFG measurements were made before, during, and after the PDT light administration. The multispectral fluorescence data were used to eliminate autofluorescence in the red wavelength band of 640±20 nm,[28-30] and produce a false-color composite image optimized for viewing the characteristic red fluorescence emission from Photofrin centered at 640 nm (Figure 1). Due to the small sample size (N=6), statistical analysis was not attempted.

Results

Histological assessment of the biopsy samples showed four patients with glioblastoma multiforme (World Health Organization Grade IV), one patient with benign cystic meningioma, and one patient with chordoma.

Table 1
Patient Data*

Patient No.	Diagnosis	Localization and lesion diameter	PDT
1	Benign cystic meningioma	Left frontal, 5 cm	No
2	Cystic astrocytoma mixed with GBM	Left frontal and temporal, 5 cm	Yes
3	Recurrent GBM	Right frontal, 5 cm	Yes
4	GBM	Right parietal, 4 cm	Yes
5	GBM	Right occipital, 5 cm	Yes
6	Recurrent chordoma	C4-C5, 6 cm	Yes

* *GBM, glioblastoma multiforme*

Figure 5A. *White light image (3.3 x 2.5 cm) of the resection cavity, with a cotton string as spatial marker.*

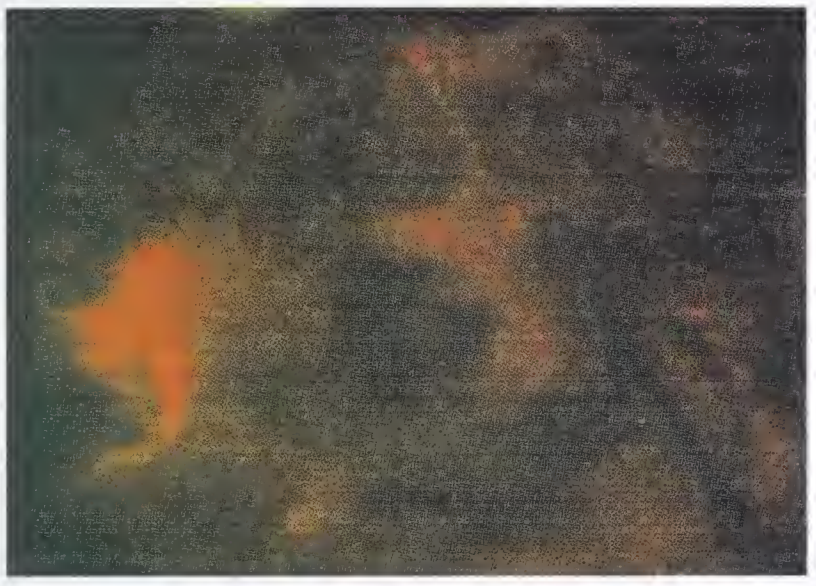

Figure 5B. *Corresponding fluorescence image showing GBM regions with bright red fluorescence. Notice the small, well-defined red regions (arrows) projecting outward.*

Intraoperatively, glioblastoma multiforme tissue in the resection cavity showed strong characteristic red fluorescence (Figure 5B). Small, well-defined papillary projections of the red fluorescence region were visible in the image, consistent with the infiltrative nature of glioblastoma multiforme. Histological analysis of the biopsy samples taken from the region with bright red fluorescence confirmed the presence of malignant tumour. *Ex vivo* Photofrin uptake measurement of this tissue showed a concentration of 9.5±0.4 μg/g. The surrounding tissue, which showed reduced red fluorescence and appeared green in the composite image due to the fluorescence emission of endogenous molecules, was normal as confirmed by histology. *Ex vivo* Photofrin uptake measurement of tissue sample taken from this region showed a concentration of 0.8±0.2 μg/g. It was evident that the contrast between the tumour and normal tissue observed in the fluorescence image was not observable in the corresponding white-light image (Figure 5A).

After surgical resection, regions with residual red fluorescence were observed in all five PDT patients. The feature size of these regions ranged from sub-millimeter to about 10 mm. After PDT treatment, residual red fluorescent regions were found in two patients (# 3, 5). These regions were biopsied and histological assessment showed tissue with vascular proliferation (# 3) and glioblastoma multiforme (# 5). Fluorescence imaging assessment of photo-bleaching was carried out on one patient (# 3), when images were obtained before, during, and after the PDT process (Figure 6A to 6F). As shown in Figure 6G, the red fluorescence intensity decayed during the treatment while the green fluorescence intensity increased.

Connective tissue rich in collagen and elastin fibers, such as bone and dura, appeared green in the composite fluorescence image. Hemoglobin attenuated the fluorescence excitation light substantially. Thus, hyperemic regions appeared dark in the fluorescence images. Metallic surgical instruments appeared dark. Cotton patties, on the other hand, fluoresced brightly in the green which was convenient to serve as spatial markers during imaging.

Discussion

The intraoperative fluorescence observation of the surgical resection fields that we obtained was in good agreement with those reported[21] using modified surgical microscopes which provided qualitative assessment of porphyrin fluorescence at a single wavelength (~ 630 nm). In comparison, the MFG system is a calibrated quantitative instrument which measures spatially and spectrally resolved fluorescence images with an error less than 10% in fluorescence intensity. Practically, the gain (or amplification) for the fluorescence signal is fixed in the MFG system. Technically, the MFG system included all components needed for absolute (as opposed to relative) measurements of the fluorescence intensity, if the target-to-detector geometry[1] can be maintained in between different patients. One of the most important parameter of this geometry is the target-to-detector distance, which is conserved between patients if the resection fields are in-focus as they appear in our images since the MFG's depth of field (~2 cm) is small compared to its working distance (50 cm). Hence, quantitative comparison between images can be made, and images obtained from different patients can be compared.

The present observations suggest that red fluorescence from Photofrin may correlate with the presence of malignant glioma tissue at submillimeter length scale when viewed under an intraoperative fluorescent detection device such as the MFG system. It is conceivable that such correlation could be used to guide surgical resection and promote better tumour tissue removal in silent areas of the brain, which could in turn improve survival rate. Residual fluorescence in unresectable or "eloquent" areas detected by the MFG system may be an indication for the use of PDT, which is less traumatic to neural tissue than surgical resection.

In the present PDT trial, the light dosimetry is empirically based on the lesion cavity size. Within the PDT treatment cavity, the current dosimetry protocol aims to achieve a uniform light dose. However, since the differential uptake of Photofrin between normal neural tissue and malignant glioma is only ~ 10 μg/g vs 1 μg/g, and preclinical studies suggest that normal brain tissue may be more sensitive to PDT than malignant tis-

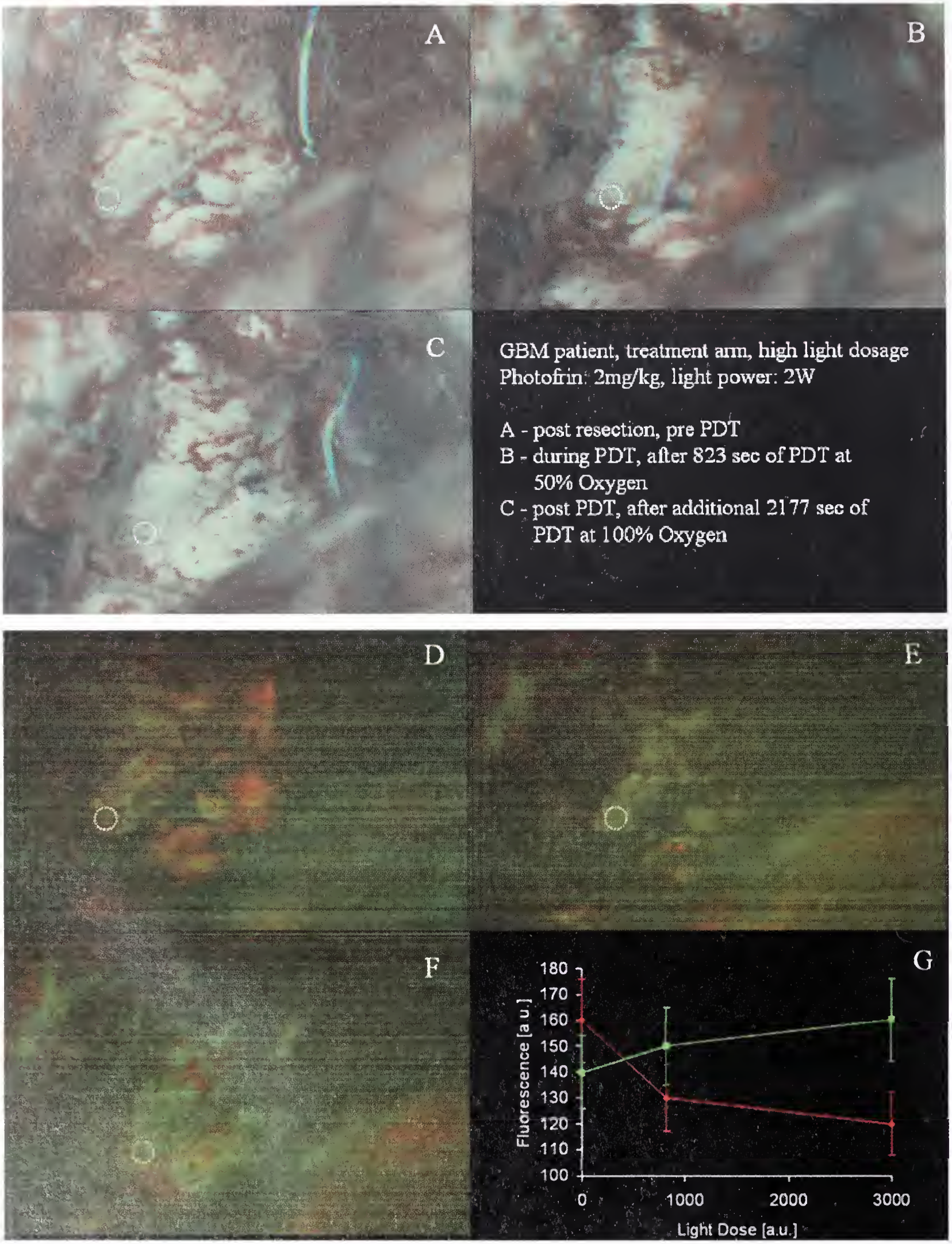

Figure 6A-F. A-C: White light time-lapse images of PDT treatment cavity. D-F: Corresponding fluorescence images showing the effect of photobleaching. G: Fluorescence intensity change as a function of treatment time, measured from the area marked as dashed circle in the images, demonstrating photobleaching of the red fluorescence (red line) and an increase in the green fluorescence (green line).

sue,[24, 32] it is possible that a spatially resolved dosimetry protocol could enhance local tumour damage while preserving surrounding normal tissue. Analogous to the conformal therapy technique commonly used in radiation oncology,[35] an optical "multi-leaf system" or a scanning optical beam could deliver different light doses to different areas in the lesion cavity. Thus, spatially resolved PDT dosimetry could potentially improve survival and reduce morbidity. It has also been proposed that intraoperative photobleaching measurement may be used for PDT dosimetry,[33] tailored to individual patient in each operation to ensure treatment completeness. Quantitative intraoperative imaging devices, like the MFG system, provide a technical possibility and platform for these advanced dosimetry protocols.

An optical intraoperative detection system such as the MFG can be obtained at a fraction of the cost of conventional intraoperative CT or MRI. In addition, the spatial resolution is superior to all CT and most MRI. One limitation of the MFG system is that the fluorescence detection method is not tomographical, thus only providing information for the most superficial layer (thickness < 1 mm) of the surgical field. However, this limitation could be overcome by using PDT as an adjuvant treatment after surgical resection since the PDT penetration depth in tumour tissue is about 5 mm.[31]

Conclusion and Future Work

Surgical fluorescence microscopy is feasible in operating rooms with specialized yet affordable equipment. Our intraoperative observations suggest that Photofrin used for PDT may serve as a fluorescent marker for malignant gliomas, which potentially allows fluorescence guided surgical removal of tumour tissue in operable areas of the brain. The residual red fluorescence in unresectable areas of the brain could serve as an indication of PDT. These could enhance the completeness of tumour removal, improve survival, and reduce morbidity in patients with malignant gliomas.

The quantitative nature of the MFG system creates opportunity for detailed *in vivo* study of the kinetics of photosensitizer during PDT. A clinical trial with larger sample size could determine the sensitivity and specificity of fluorescence detection of malignant glioma, and provide a quantitative fluorescence intensity threshold to distinguish normal tissue from tumour. Such threshold experiment is necessary before the fluorescence technique can be applied to aid in determining resection margin intraoperatively.

The multispectral ability of the MFG system allows easy adaptation to other applications in open field surgery. It also creates the opportunity of using fluorescence tagged monoclonal antibody to guide surgical resection. A MFG system has been tested for *in vivo* detection of oral cancer as part of our collaboration with Dept. Oral and Maxillary Surgery, State University of New York at Buffalo (unpublished data). A MFG system will also be used in a clinical trial of intraoperatively guided surgical removal of ductal carcinoma *in situ* in Toronto.

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Recent developments in amebiasis: The Gal/GalNAc lectins of Entamoeba histolytica and Entamoeba dispar

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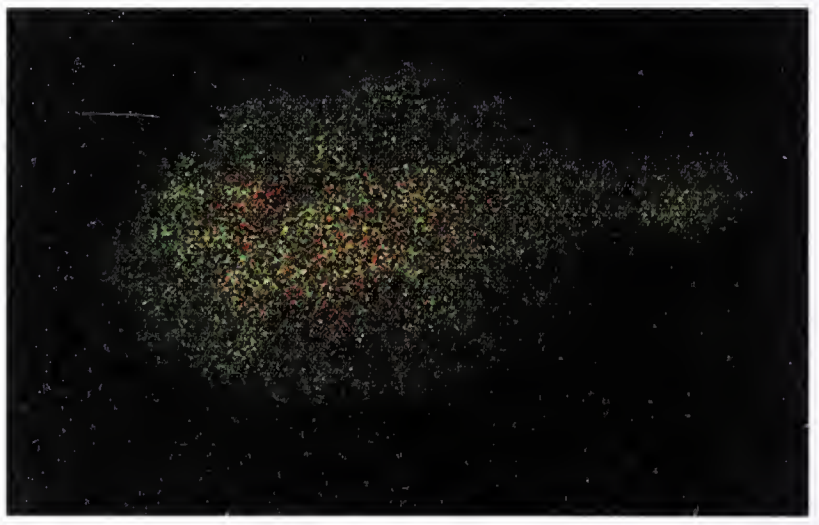
Abstract

Amebiasis is responsible for 50,000 – 100,000 deaths annually. Invasive amebic disease begins with the attachment of *Entamoeba histolytica* trophozoites to colonic mucin, a process mediated by the amebic Gal/GalNAc lectin. The non-pathogenic counterpart, *E. dispar*, is morphologically identical but genetically distinct. Investigations are underway comparing the Gal/GalNAc lectin from these two organisms.

E. histolytica Lectin: Adhesin and Virulence Factor

The amebic cell surface lectin was first implicated in adherence and contact-dependent cytolysis of human cells by Ravdin and Guerrant in 1981.[1] Centrifugation binding assays have demonstrated that amoebae interact with human colonic mucin, human colonic epithelium, human neutrophils, and erythrocytes in a galactose- and *N*-acetylgalactosamine-inhibitable manner. Assays are performed at 4°C in order to isolate the adherence properties of the amoeba since trophozoites will lyse most eukaryotic cells at physiologic temperatures. Furthermore, Chadee and workers demonstrated that purified native lectin bound human colonic mucin with very high affinity (8.2×10^{-11} M^{-1}).[2] Mucin likely represents the primary interface of amoeba and human colon during colonization.

Elegant studies determining the sugar-specificity of the amoeba and target cells relied on Chinese Hamster Ovary (CHO) cell glycosylation pathway mutants.[3-5] The striking observation was made that a particular CHO cell line (1d1D.Lec1), which lacks *N*- and *O*-linked galactose and *N*-acetylgalactosamine sugars, adhered to amoeba 95% less than wild type CHO cells. Interestingly, cytotoxicity was also diminished by 90%, suggesting that lectin-sugar interactions are necessary for contact-dependent killing of target cells in this assay. Cytotoxicity refers here to the artificial apposition of amoebae and ^{51}Cr-loaded target CHO cells, followed by lysis and measurement of ^{51}Cr release. Further evidence that cytotoxicity was contact-dependent came by way centrifugation assays performed in 10% dextran, which prevented apposition of amoebae and CHO cells, and resulted in significant inhibition of adherence and cytotoxicity.[1]



Confocal image of *E. histolytica* trophozoite using dual-colour indirect immunofluorescence of ß2-integrin (red) and Gal/GalNAc lectin (green).

Isolation of purified native hololectin from amebic trophozoites was achieved by gel filtration analysis. A broad elution peak of approximately 660-kDa suggested that the amebic lectin could form oligomers.[6] Confirmation that the eluted protein was consistent with the lectin was made by: (i) galactose-specific CHO cell binding; (ii) recognition by monoclonal antibodies (MAbs) subsequently generated against the recombinant heavy subunit; (iii) functional studies showing that anti-native lectin antisera, but not pre-immune sera, blocked adherence to CHO cells. Despite commonalities with the C-type lectin, asialoglycoprotein, the amebic lectin does not contain primary sequence compatible with well-described carbohydrate recognition domains (CRDs). The

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amebic lectin is a 260-kDa heterodimer, composed of a 170-kDa heavy subunit and a 31/35-kDa light subunit which is glycosylphosphatidyl-inositol (GPI)-anchored into the surface membrane bilayer (Figure 1). At least five genes encode the heavy subunit, three of which have been fully sequenced.

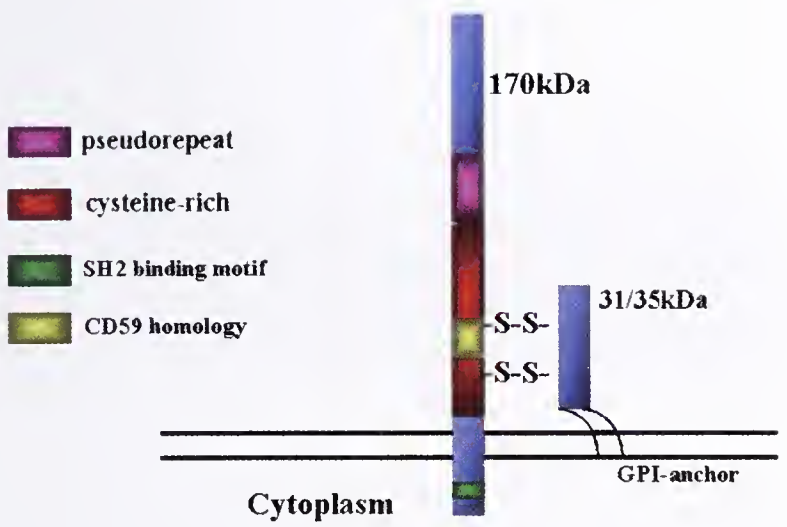

Figure 1. *Subunit structure of the E. histolytica Gal/GalNAc lectin.*

The predicted amino acid sequences of *hgl1* and *hgl3* are 95.2% identical, and share 89.2% and 89.4% identity with *hgl2*, respectively.[7,8] The heavy subunit has several interesting features: (i) The amino terminal (residues 1-187) has an abundance of cysteine (3.2%) and tryptophan (2.1%) residues. (ii) Amino acids 187-378 are characterized by the absence of cysteines. (iii) A repeating region of ~30 amino acids was identified between residues 379-654. (iv) Residues 379-1209 have an extraordinarily high number of cysteines (97 or 10.8%) and a domain which bears significant identity to the mammalian CD59 protein associated with complement resistance. (v) Hydrophobicity plots suggest residues 1210-1235 likely constitute the single membrane-spanning region. (vi) The remaining carboxy-terminal residues (1236-1277) contain an epidermal growth factor receptor (EGFR)-like tyrosine phosphorylation motif (Asn-Glu-Asn-Ala-Glu-Tyr). (vii) Potential *N*-linked glycosylation sites (16 in *hgl1* and *hgl3*, 9 in *hgl2*) are present throughout the protein.

The small subunit has a calculated molecular weight of 32-kDa with hydrophobic amino- and carboxy-terminal signal sequences.[9-11] Both *hgl1* and *hgl2* (80.1% identical) contain putative *N*-glycosylation sites and possess no significant homologies with other known proteins. However, a GPI anchor cleavage or addition site was identified. The light subunit exists as either a 31-kDa GPI-anchored or 35-kDa non-anchored isoform on the native lectin of amoeba. Nitrous acid deamination of the [^{3}H]palmitate-labelled 31-kDa, but not the 35-kDa, isomer resulted in acyl-phosphatidyl inositol release, supporting the presence of a GPI anchor. The disparity in molecular weight for the native light subunits likely arises from variation in lipidation and glycosylation. It is unusual that the 31-kDa isomer contains both a putative transmembrane domain and a GPI anchor.[10] The role of the light subunit remains elusive. Recent developments in antisense inhibition of *E. histolytica* gene expression by Mirelman and colleagues showed that suppression of light subunit gene expression results in diminished amebic cytotoxicity but not adherence.[12] These data would suggest the light subunit either facilitates hololectin expression or has direct cytotoxic effects through an undefined mechanism.

Fine-mapping the Carbohydrate Recogntion Domains (CRDs) of the *E. histolytica* Gal/GalNAc Lectin Heavy Subunit

A breakthrough in understanding amebic lectin sugar affinity was made when Schnaar and colleagues demonstrated that *E. histolytica* membranes bind to the glycoconjugate, $GalNAc_{39}BSA$, with over 10^5-fold greater affinity than GalNAc alone.[13] This result was intriguing because it suggested that the amebic lectin was capable of achieving very high affinity association by either (i) the additive effect of multiple lectin-sugar interactions and/or (ii) the additive effect of several CRD-sugar interactions within a single lectin molecule.[14] However, these experiments relied on amebic membranes and therefore the question of which of the two lectin subunits (170-kDa or 31/35-kDa) possessed CRD(s) remained unresolved. Previously, MAbs directed against the heavy subunit were shown to be adherence inhibitory in the standard amoeba-CHO cell adherence assay, suggesting the CRD(s) lay within the 170-kDa subunit.[15] However, this did not provide sufficient proof since antibodies are large molecules (~160-kDa) and can exhibit steric hindrance (non-specific) of cellular adherence.

Petri and colleagues have provided direct evidence for a CRD located between residues 895-998 of the 170-kDa subunit.[16] These workers bound radiolabelled $GalNAc_{19}BSA$ to recombinant lectin produced in a bacterial expression system. Recombinant fragment 895-998 was imunoreactive with MAb 8C12 and bound $GalNAc_{19}BSA$ in a GalNAc-inhibitable manner, suggesting the presence of a CRD. This assay relied on radiolabelled glyconeoconjugates binding to recombinant lectin fragments blotted on to PVDF membranes followed by densitometric analysis of autoradiographs. Estimation of the association constants for this fragment compared to the entire heavy subunit would help determine whether 895-998 represented the sole CRD. If the affinity of the smaller fragment was lower, it would suggest the presence of several CRDs within the 170-kDa subunit. Region 895-998 is in a juxtamembrane position (TM=1210-1235) and is presumably accessible to terminal sugars expressed in colonic mucin. These data also suggest binding is conformation-insensitive since recombinant fragments were run on SDS-PAGE gels under reducing and denaturing conditions, and still remained functional. For future studies, a crystal solution structure of a bacterially-expressed CRD (895-998) with Gal or GalNAc would facilitate a detailed understanding the binding mechanism of the amebic lectin.

Fragments of the lectin (heavy and light subunit) were also synthesized *in vitro* with ^{35}S-amino acid incorporation using rabbit reticulocyte lysate.[17] Since *in vitro* transcription and translation yields minute quantities of protein, a very high-affinity ligand was required in order to generate binding isotherms and generate affinity constants. Glyconeoconjugates bound to lectin in the nM range and were therefore suitable candidate ligands.[13] Commercially available glyconeoconjugates were immobilized onto microwells and were shown to specifically bind region 356-1143 (cysteine-rich region) of the lectin heavy subunit, but not other fragments (1-480) of the heavy subunit.[17] A smaller fragment (480-900) within the cysteine-rich region did not bind to the same maximal activity as 356-1143. The binding affinity of the 356-1143 was in the pM range, suggesting a very high affinity association characteristic of multivalent interactions. It is unclear whether the high affinity achieved in this *in vitro* assay is due to the presence of several CRDs within the heavy subunit or the requirement for discontinuous residues to provide the correct conformation for the high-affinity state.

These data beg further questions: (i) Do discontinuous residues between 356-480 or 900-1143 contribute to high-affinity carbohydrate binding by forming a conformation-sensitive binding pocket – "conformational model"? (ii) Do several CRDs, which lie within the heavy subunit, provide several low affinity interactions that combine to provide an avid interaction with multivalent glyconeoconjugates – "subsite multivaleney model"? (iii) Do several lectin dimers cluster at the cell surface to provide a high avidity interaction – "subunit multivalency"? (iv) Does a combination of the above occur? Evidence both supporting and refuting these hypotheses exists in the literature.

On the one hand, it was reported that two MAbs directed against the heavy subunit (3F4, 8A3), enhaneed adherence in the amoeba-CHO cell adherence assay. Since this assay is performed at 4°C, it is likely that these antibodies induee a high-affinity binding conformation on the lectin rather than reeruiting more reeeptor to the surface. Sueh "ligand-induced binding" has been observed in mammalian leukocyte adherence.[18] An alternative interpretation of the activating-antibodies, 3F4 and 8A3, is that they cause clustering of the lectin on the amebic surface leading to spatial rearrangement of lectin molecules that affords high-affinity interaction in whole cell adherence assays. This assumes that leetin-mediated signalling to the amebie cytoskeleton is possible at 4°C, the temperature at whieh the antibody effects were observed. Previous work has implicated the leetin in aetin-mediated eytoskeletal ehanges.[19] Mathematical modeling of binding data obtained with glyeoneoeonjugates suggested that enhaneed binding phenomena assoeiated with polyvalent ligands, like GalNAe$_n$BSA, may be explained by the additive effects of simple multivalent interactions.[20] This would suggest that subsite (if there are more than one CRDs per leetin moleeule) and subunit multivaleney (when using whole membranes) are more significant than conformation of the lectin.

The relevance of high-affinity adherence to glyeoneoeonjugates is made apparent when one considers the abundance of mucin (a naturally-oeeurring glyeoeonjugate) in the large intestine. Chadee and colleagues demonstrated that *E. histolytica* trophozoites bound rat intestinal mucin and human colonic mucin with very high-affinity (pM range) through engagement of the Gal/GalNAe lectin.[2] Mucin likely plays a cytoprotective role (together seeretory IgA and defensins) for intestinal epithelial cells during asymptomatic colonization of the bowel.[21] During invasive disease, amebic mueosal erosion occurs accompanied by mucosal entry into the crypts, but not the lamina propria.[22] Epithelial destruction ensues leading to neutrophil recruitment and activation of localized inflammation. Since mucin attaehment appears to preeede downstream eytolytic and inflammatory events, it is conceivable that the generation of agents (antibodies or glycoconjugates) eapable of specifically disrupting (and not enhancing) the leetin-sugar interaction may prove useful as a therapeutie intervention. To this end, a detailed understanding of the conformational regulation and/or subsite multivalency of CRDs within the heavy subunit may permit the rational design of new therapies for amebiasis.

Implications of Structure-Function Similarities and Differences Between the *E.histolytica* and *E.dispar* Gal/GalNAc Lectin

Perhaps the most profound development in *Entamoeba* research has been the near universal acceptance of the commensal *E. dispar* as a distinct speeies. Sargeaunt and colleagues demonstrated that isoenzyme profiles (zymodemes) correlated with virulence in the human host.[23] Non-pathogenie zymodemes were collectively renamed "*Entamoeba dispar,*" and pathogenic zymodemes, "*Entamoeba histolytica,*" in keeping with Emile Brumpt's prescient observations in the 1920s.[24] Confounding matters, however, have been sporadie reports by investigators in endemie countries that so-ealled *E. dispar* zymodemes have been observed in patients with symptomatie disease.[25] In addition, data presented by Mirelman and colleagues have supported the conversion of *E. histolytica* and *E. dispar* trophozoites cultured *in vitro*.[26]

The epidemiology of *E. histolytica* and *E. dispar* infection in a non-endemic setting was addressed by Weinke and colleagues in Berlin, Germany.[27] These investigators followed over 2700 travelers and 320 active gay males (both high risk groups) and showed that 16.3% of the gay male cohort and 4.0% of the travelers had *E. histolytica* infections. Amongst infected travelers, 92.5% were *E. dispar* infections by zymodeme analysis, and all 100% of infeetions in gay males (presumably due to local, direct transmission) were due to *E. dispar*. In a similar study conducted in Toronto, of 112 individuals with risk factors for E. histolytica infection, 72 were aetually infected, and of those 95.8% were due to *E. dispar*.[28] Therefore, while the inter-conversion of *E. histolytica* and *E. dispar* in culture remains controversial, it is becoming clear that the vast majority of *Entamoeba* human infections, particularly in developed countries, are due to an organism that appears to be a harmless commensal. A small minority (<10%) is infeeted with *E. histolytica sensu stricto*. Interestingly, infection with the pathogen does not guarantee an invasive episode since individuals appear to be able to tolerate infection for years without clinical disease.[29] These kinds of epidemiologieal and clinical scenarios raise the question: are structure-funetion differences in E. *histolytica/dispar* virulenee factors, such as the Gal/GalNAc lectin, informative in understanding the differential virulence *in vivo*?

For some time, a technical diffieulty precluded a strict compare and contrast study between *E. histolytica* and *E. dispar*, namely that *E. dispar* appeared to require co-culture with bacteria (xenic culture) in order to survive, whereas *E. histolytica* is able to survive in the absenee of other organisms (axenic eulture). However, Seiki Kobayashi (Keio University, Tokyo) and Graham Clark (London School of Hygiene & Tropical Medieine) have recently developed axenic media conditions for *E. dispar* culture.[30,31] Using a PCR amplifieation approach with cDNA prepared from an axenic culture of strain SAW 760, a gene homologous to *E. histolytica hgl2* was identified in E. dispar.[32] Complete sequence analysis, Southern blot hybridizatrion, and RT-PCR indicated that this gene was not present in *E. histolytica*, was actively transcribed, and possessed a high degree of sequence identity (87% at the amino acid level). Comparison of secondary structure predietions for Dhgl2 and Hgl2 demonstrates regions of α-helix, ß-sheet, and ß-turn conservation (*N* terminus) as well as divergence. Interestingly, there is greater predieted α-helieal secondary structure in Hgl2 in the extraeellular, juxtamembrane region (see arrow, Figure 2). Homology modelling and threading are not possible for the amebic lectin since these genes bear no sequence similarity to crystallized lectins. A restriction fragment length polymorphism (RFLP) in the lectin homologues (*hgl2* and *dhgl2*) permitted a straightforward PCR-RFLP approach to distinguishing *E. dispar* from *E. histolytica*, which may be of diagnostic value.

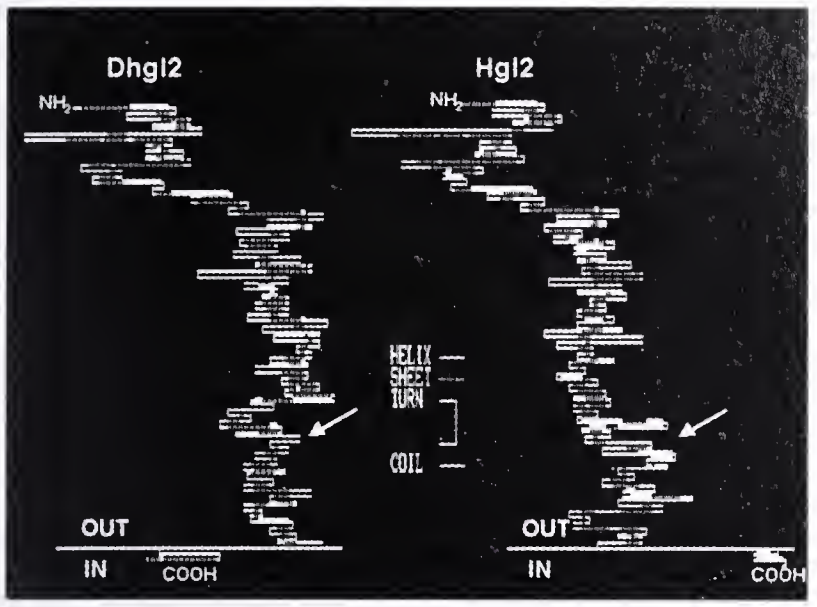

Figure 2. *Schematic representation of Chou & Fasman plot of amebic lectin heavy subunit homologues from E. dispar (Dhgl2) and E. histolytica (Hgl2). Secondary structure prediction demonstrates regions of a-helix, ß-sheet, and b-turn conservation (N terminus) as well as divergence (greater predicted a-helical secondary structure in Hgl2 in the extracellular, juxtamembrane region, see arrow).*

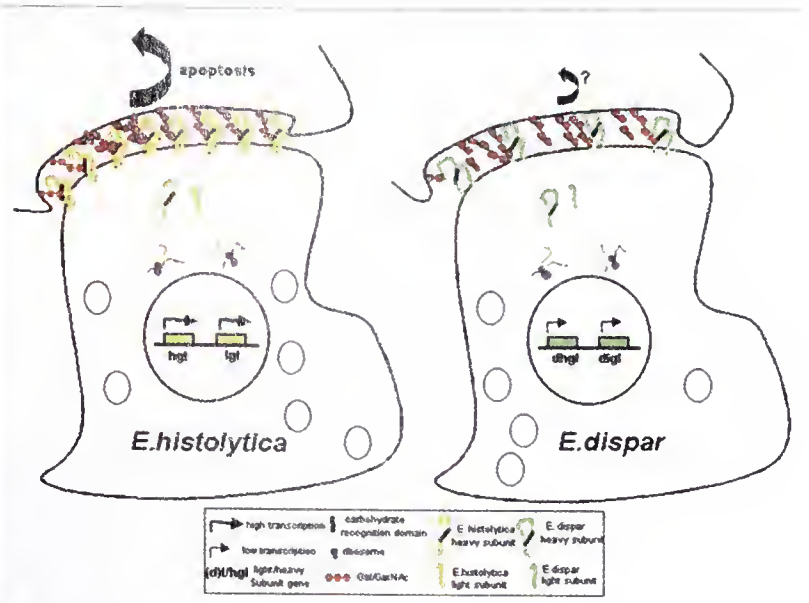

Figure 3. *Working hypothesis of E. histolytica and E. dispar Gal/GalNAc lectin. Models of lectin-dependent adherence for E. histolytica and E. dispar are depicted. Transcript levels and surface expression are higher in E. histolytica. The heavy subunit CRD may be constitutively exposed on the surface of E. histolytica but not E. dispar. Intimate contact of target host cells leads to apoptosis either through receptor-mediated caspase activation or secondary to amoebapore-mediated cytolysis and Ca^{2+} flux in target cells.*

Radioimmunossay experiments with *E. histolytica* and *E. dispar* trophozoites demonstrated epitope conservation (3F4, 8A3) as well as divergence (1G7, 7F4, 8C12) to a panel of MAbs directed against the heavy subunit.[33] While 3F4 and 8A3 recognized the native lectin of *E. dispar*, 1G7 did not detect this protein under native conditions on the cell surface. However, the *E. dispar* lectin heavy subunit (480-900) was 1G7 positive when recombinantly expressed in COS7 cells (manuscript in preparation). Since the 1G7 epitope maps to 482-818, it falls within the cysteine-rich fragment 480-900 shown earlier to bind $GalNAc_{19}BSA$ and by inference contains one or more CRDs.[17] This may have important functional implications since lectin-mediated adhesion and "de-adhesion" could be achieved by modulation of conformation. Mann and colleagues performed *E. dispar* trophozoite-CHO cell adherence and cytolysis assays, and reported that the *E. dispar* adhered to and lysed target cells to a lesser extent than *E. histolytica*.[34] It is tantalizing to suggest that the concealment or folding away of the 1G7 epitope on the native *E. dispar* lectin may explain the lower affinity for GalNAc in whole cell adherence assays, as well as diminished *E. dispar* virulence in small animal models.[34,35] Since *in vitro* binding assays of the highly homologous heavy subunits (Dhgl2 and Hgl2) demonstrated that both *E. histolytica* and *E. dispar* lectins bound $GalNAc_{19}BSA$ with equivalent affinity, it is more likely that conformational differences rather than intrinsic sugar-affinity differences account for difference in cellular adherence (manuscript in preparation). A discrepancy in the relative amounts of lectin expressed on the amebic surface could also account for different adherence properties of *E. histolytica* and *E. dispar*. Flow cytometric analysis showed that *E. histolytica* expresses more cell surface hololectin (heavy and light subunits) than *E. dispar* when both organisms are cultured in the absence of other liver organisms (manuscript in preparation). Therefore, we propose a model where greater lectin gene expression and conformational regulation to a high-affinity state may contribute to tight binding of carbohydrate by *E. histolytica* (Figure 3).

E.histolytica Infection and the Other Virulence Factors

The primary candidate adhesion molecule, which likely mediates adhesion to intestinal mucin, is the galactose/*N*–acetylgalactosamine lectin.[2] However, other candidate virulence factors have been posited: (i) Degeneration of epithelial cells, dissolution of the basement membrane, and degradation of host immune effector molecules (IgG and IgA) are likely mediated by extracellular cysteine proteases, several of which have been cloned, characterized, and implicated in invasive disease. (ii) A family of pore-forming amphipathic peptides (amoebapores) have been identified in *E. histolytica* and implicated in its potent cytolytic capacity. Prior to traversing the bowel wall, it is likely that these enzymes degrade host extracellular matrix (ECM) as well as the basement membrane. Evidence for this has been presented by correlating the expression levels of the cysteine proteases with the cytopathic effect amoebae have on mammalian target cell monolayers.[36-40] Mutant *E. histolytica* strains deficient in cysteine protease activity were diminished in their capacity to disrupt target monolayers. Specific cysteine but not serine protease inhibitors attenuated the cytopathic effect of trophozoites on the same monolayers. Furthermore, purified amebic cysteine proteases were able to mimic the amoeba-driven cytopathic effect on monolayers. While it is speculated that these proteases also degrade laminin and fibronectin, major ECM proteins found in association with the intestinal epithelium, no direct *in vitro* evidence has been provided.

Several reports suggest that amoeba-derived cysteine proteases may exacerbate inflammation and tissue damage associated with granuloma and abscess formation in animal models. Protease inhibitors were able to decrease tissue damage in rats that had been subcutaneously inoculated with amebic trophozoites. In another study, severe combined immunodeficiency (SCID) mice, which lack both B and T cells due to a genetic defect in hematopoietic development, were challenged intrahepatically with trophozoites.[41] These workers noted that preincubation of amoebae with the highly specific cysteine protease inhibitor, E-64 [L-*trans*-epoxysuccinyl-leucylamido-(4-guanidino) butane], was able to reduce abscess size in the liver when assessed 48 hrs post-challenge.[42] Additionally, a MAb directed against amebic cysteine pro-

tease demonstrated that this enzyme is expressed within the abscess of infected animals. Co-injection of amoebae and laminin also reduced abscess size, suggesting laminin served as a competitive substrate for protease activity.[43] The extracellular location of these proteases was further supported by evidence that 83% of patients succumbing to invasive disease mount an antibody reponse specific to the amebic cysteine protease.[44] A detailed analysis of the cysteine protease family has identified a surface bound member (CP5) that is present and expressed in *E. histolytica*, but may be degenerate in *E. dispar*.[45,46,47] Cytolysis of mammalian cells by *E. histolytica* trophozoites is a rapid event initiated by intimate contact of effector and target cell. Within a matter of minutes, swelling and widespread surface blebbing occurs in the target. While initial adherence is likely mediated by the *E. histolytica* galactose/N-acetylgalactosamine lectin, proteinaceous factors are released which compromise the target membrane. Failure to exclude dyes such as propidium iodide clearly demonstrated target cell death. Evidence for apoptotic and necrotic target cell death exist, and has to be fully resolved.[48–51] More precisely, it is unclear if target cell death is a function of receptor-mediated apoptosis (analogous to Fas/Fas Ligand) and/or membranolytic peptides (analogous to granzymes).

The first mechanistic evidence for the cytolytic event was the discovery that amoeba are capable of inducing a pore in the lipid bilayer of the target, which resulted in depolarization of the cell.[52,53] Subsequent protein purification and genetic cloning revealed that this protein was a pore-forming peptide, comprised of at least three family members.[54,55] Termed "Amoebapore" A, B and C, these genes encode a 77 amino acid mature peptide with 6 cysteine residues and 1 histidine residue in conserved positions. The three isoforms retain 35-57% overall identity based on amino acid sequencing and mass spectroscopy.[56] A single trophozoite was shown to express all three amoebapores in a ratio of 35(A): 10(B): 1(C).[55] Amoebapores share significant sequence identity (25-30% based on exact amino acid identity and 45-50% based on functional amino acid identity) with NK-lysin from mammalian natural killer (NK) and T cells.[57] Structural analysis using circular dicroism spectroscopy indicated that amoebapore A has a compact α-helical structure. Secondary structure prediction has demonstrated that the polypeptide chain folds into four, principally amphipathic, helical elements connected by 3 amino acid loops.[55] The amoebapore is also refractory to proteolytic cleavage (except for the loops) and denaturation, and retains no free thiol groups. These data suggested that this amphipathic pore-forming peptide was highly compact and stable, consistent with its 4-α-helix bundle predicted stucture. A similar predicted structure was obtained for NK-lysin using the same experimental approach, and is unlike other cytolytic peptides.[58] In parallel with findings for the lectin, *E. dispar* retains homologues for all amoebapore genes present in *E. histolytica*, but the non-pathogenic peptides appear to adopt an altered structure based on homology modelling.[59]

From the parasite's eye view, water-borne passage requires consistent release of cysts into the water, the commonest scenario in the developing world. Due to water filtration systems in non-endemic settings direct transmission, fecal-oral contact in gay males or unhygienic conditions in institutionalized settings, are the only apparent mode of infection.[60] Retention of cytolytic capacity may be selected for evolutionarily only in so far as they allow amoebae to feed on enteric bacteria and defend against host immune effector functions.[61] Since disruption of the intestinal epithelium, entry into the portal circulation, and seeding of the liver confers no evolutionary advantage to the parasite and often kills the host who cannot then pass on infective cysts, extra-intestinal amebiasis likely occurs "by accident." Hence, many so-called "virulence factors" described in the *Entamoeba* lit-

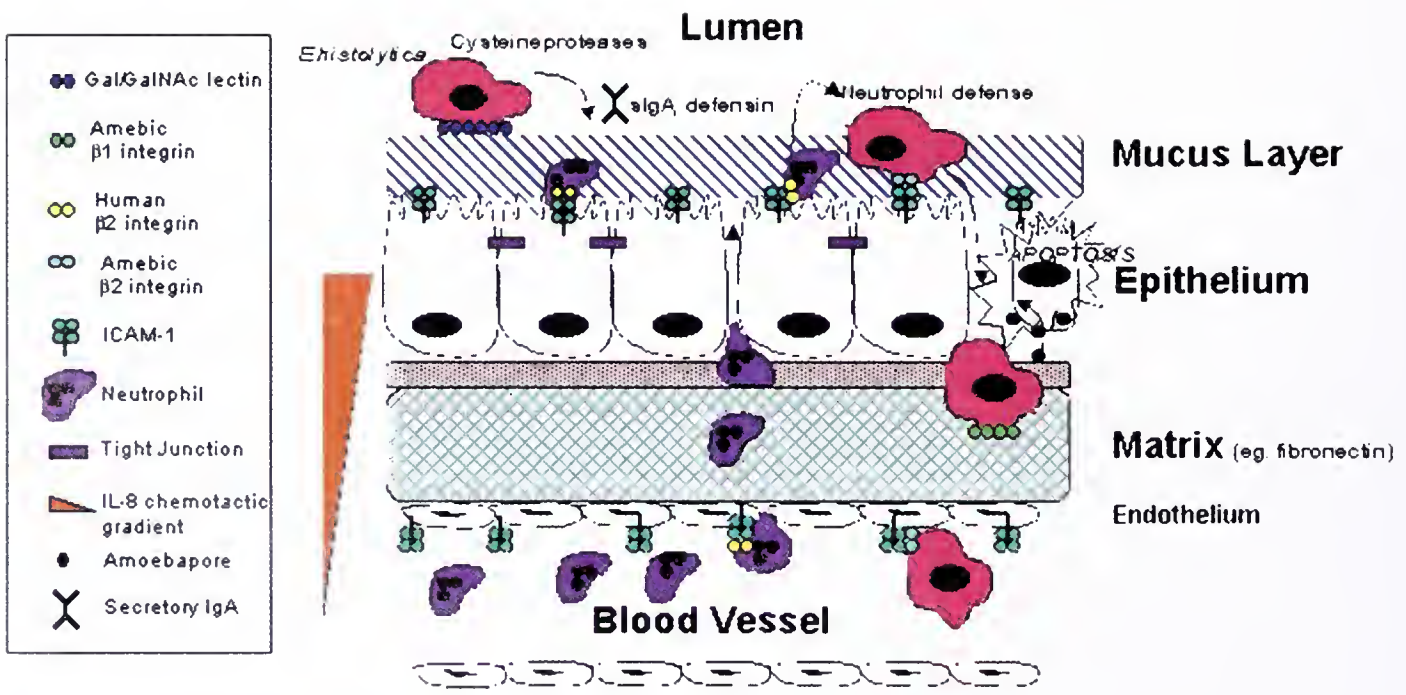

Figure 4. *A model where trophozoites adhere to mucus in the large intestine via clustered Gal/GalNAc lectin is presented. Colonization of the bowel following initial adherence may be facilitated by the degradative action of cysteine proteases on mucin, IgA and other soluble factors. Localized inflammation and mucosal ulceration allows deeper penetration of trophozoites that may trigger cytokine release from and apoptotic death of intestinal epithelial cells. Interleukin-8 is induced by E histolytica secretory factors from colonic cell lines in vitro and in intestinal amebiasis models. IL-8 recruits neutrophils in part by up-regulating ICAM-1. This may allow trophozoites (expressing putative ß2 integrin) to attach to intestinal epithelial cells and penetrate into the submucosa. Here, trophozoites can traverse ECM proteins like fibronectin via amebic ß1 integrin, and subsequently attach to endothelial cells (where ß2 integrin-ICAM-1 interaction may also occur) and enter the portal circulation*

erature may in fact be housekeeping proteins gone awry. Clinical epidemiological data support the above view in the sense that only a small fraction (1 per 1000) of those infected with pathogenic *E. histolytica* actually succumb to extra-intestinal symptoms, and of those many are immunocompromised during pregnancy, poor nutritional status, or immunosuppressive drug therapy.[62–64]

The lectin appears to mediate adherence to most host cell types.[1,65] However, it would be difficult to rationalize the ability of this motile parasite to traverse intestinal epithelium, extracellular matrix (ECM), as well as endothelium with a single adhesion molecule. In support of this, Talamas-Rohana and colleagues have purified and characterized an amebic ß1 integrin that binds to fibronectin, an ECM constituent.[66] Binding of fibronectin leads to protein kinase C or adenyl cyclase activation, resulting in protein phosphorylation, increased actin gene expression, and cytoskeletal rearrangements that facilitate focal adhesion. Guillen and colleagues have identified a hyaluronic acid-binding protein expressed on the amebic surface that is antigenically similar to CD44.[68] Initial studies with anti-integrin antibodies had suggested the presence of ß2 integrin (CD18)-like epitope present in *E. histolytica*.[69] Further investigation of the ß2 integrin-like protein has indicated that it is expressed on *E. histolytica* and *E. dispar*, and possibly required for adhesion at intestinal sites (manuscript in preparation). These data are consistent with a model where *E. histolytica* trophozoites opportunistically adhere to up-regulated ICAM-1 during parasite-induced inflammation that occurs at intestinal epithelium or vascular endothelium. Interestingly, immunohistochemical studies have shown up-regulation of ICAM-1 on endothelium adjacent to hepatic abscess sites.[70] Chadee and colleagues have shown that trophozoites stimulated IL-8 in several colonic epithelial cells – which is known to up-regulate ICAM-1 – and thereby facilitate neutrophil transepithelial migration.[71] ICAM-1 may allow trophozoites to tether at luminal surfaces of intestinal epithelia or afford migration out of the lumen into subepithelial layers. While no clear data exist, this information is testable using *in vitro* transepithelial migration assays.[72] A putative model where the amebic lectin may act in concert with cysteine proteases, amoebapores, and integrins, is presented (Figure 4).

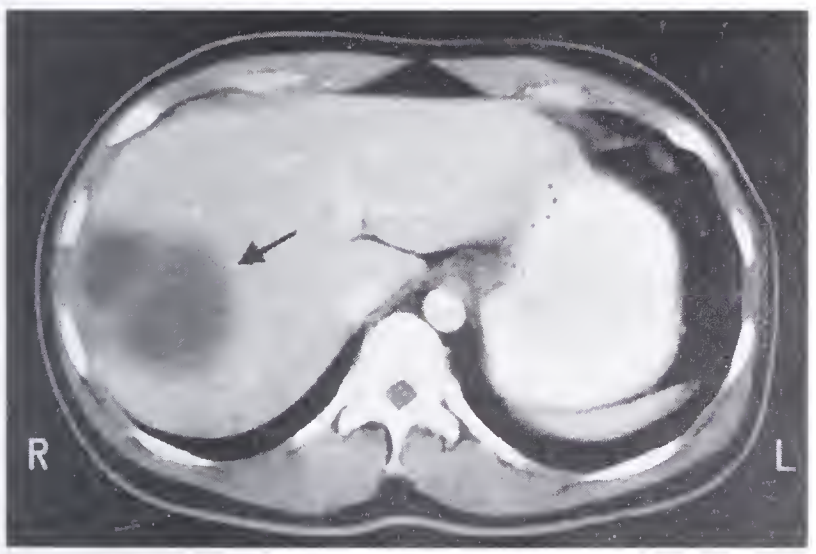

Figure 4. *Amebic liver abscess based on computed tomography (CT) scan of a 30 year-old immigrant from an endemic area.*

Vaccinology: The Utility of a Mucosal DNA Vaccine to Prevent *E.histolytica* Infection

The separation of the human immune system into systemic (bone marrow, spleen, and lymph nodes) and mucosal (gastrointestinal (GI), genitourinary, and respiratory) compartments is well accepted.[73] While both compartments do interact, this separation allows us to better understand what may constitute protective immunity when considering a mucosal pathogen, like *E. histolytica*. Whereas mucin, defensins, and flushing of lumenal pathogens through osmotic changes at the intestinal epithelium constitute innate immunity to pathogens, the induced mucosal immune system has both humoral (IgA) and cell-mediated ($CD4^+$ T helper lymphocytes and $CD8^+$ cytotoxic T lymphocytes (CTLs)) components.[74] The major inductive sites in the GI tract are the gut-associated lymphoid tissue (GALT), such as the Peyer's patches (PP), appendix, mesenteric lymph nodes, and intraepithelial lymphocytes. The cellular architecture of the PP is optimized for antigen uptake, presentation, and induction of antigen-specific affinity maturation of IgA^+ B cells from IgM^+ B cells under the influence of appropriate cytokine milieu and T cell help ($CD4^+$).[75] CTLs that recognize microbial antigens presented in the context of MHC class I molecules are particularly effective against intracellular pathogens. These antigen-specific B and T cells migrate from inductive sites to effector sites via a systemic route (mesenteric lymph nodes –> thoracic duct –> blood –> lamina propria). Mucosal defense mediated by IgA can occur in the lumen where secretory IgA (sIgA) can neutralize microbes, within epithelial cells where dimeric IgA can bind intracellular antigen, and within the lamina propria where dimeric IgA complex with antigen and transported into the lumen.

The role of mucosal immunity in combatting *E. histolytica* infection of the gut is undoubtedly crucial in preventing invasive disease. In spite of this, the most commonly used animal model (the Mongolian gerbil) for vaccine efficacy studies relies on intrahepatic challenge, which bypasses the role of mucosal immunity.[76] A suitable model for vaccine studies of intestinal amebiasis has not been developed to date. This model would require oral challenge of infective cysts either in feed or nasogastric tube, or rectal challenge of infective cysts or trophozoites (since stomach acids would not be an impediment with this route). A mouse model would be preferred since reagents to assess immunological changes are widely available for this species when compared to gerbils. Stanley and colleagues have managed to engraft fetal human intestine into a SCID mouse host in order to evaluate human intestinal responses to trophozoite challenge.[79] A small animal model in which successful colonization of the gut with *E. histolytica* occurs likely requires either transient immunosuppression, reduction of the enteric flora, strain-specific or cytokine gene knockout -specific susceptibility.

The majority of mucosal vaccines employed currently in humans are live attenuated organisms, such as oral polio, *Salmonella typhi* Ty21a, and *Vibrio cholerae*. In general, live attenuated pathogens can revert and pose a health risk, especially to immunocompromised individuals. Antigens are poor mucosal immunogens in the absence of adjuvants such as cholera toxin (CT) or heat-labile enterotoxin (LT) likely due to antigen degradation prior to reaching inductive sites. Given World Health Organization (WHO) criteria for vaccines which include affordability, heat stability (no requirement for cold-chain), single dose administration preferably mucosally, and multiple specificity, DNA immunization remains a desirable option. McCluskie and Davis recently reviewed the advantages of mucosal DNA vaccines.[80]

DNA immunization has proven successful in several models, especially where cytotoxic $CD8^+$ T cell (CTL) responses are required.[81] Recent developments in our understanding of the immunobiology of *E. histolytica* infection suggest that a Th1-like (cellular) immune responses, characterized by the requirement for elevated IFN-γ and IL-2, is crucial to host defense.[82] Th1-promoting cytokines (IL-12) combined with mucosal adjuvants (such as bacterial toxins or newer compounds like the purified saponin, QS21) can be co-immunized to potentiate the immune response. Other workers have shown that co-immunization of DNA constructs encoding the immunogen of choice coupled to either GM-CSF or B7.2 can vastly increase the potency of the cytotoxic response.[83] Furthermore, the route of administration (eg. intranasal, intraoral, or intrarectal) or method of inoculation (eg. gene-gun immunization of mucosal surfaces or liposome delivery systems) can improve specific immune responses in a given compartment. DNA immunization administered systemically is useful in priming the immune system prior to boosting with viral protein immunization.[84,85] For example, priming with DNA systemically (intramuscular injection) followed by intranasal administration of replication-deficient adenoviral recombinants expressing the antigen of choice has been shown to bolster vaginal IgA and IgG2a responses.[86] The priming effect was specific to DNA immunization and could be enhanced by IL-4 genetic adjuvants. These data suggest that DNA can also be coupled to other vaccination strategies to induce mucosal immunity at distal sites. These studies are now possible with the Gal/GalNAc lectin gene as DNA immunogen since expression of this protein in mammalian cells has been achieved with high efficiency *in vitro* (manuscript in preparation). Proof of principle will require demonstration of anti-lectin humoral responses (systemic and mucosal) able to neutralize adherence of amoebae and host cells, lymphocyte proliferation in response to amebic lectin, induction of a Th1-like repertoire of cytokines.

Specific Diagnosis of *E.histolytica* and Not *E.dispar*: Clinical Implications

Distinguishing *E. histolytica* from *E. dispar* in the clinic can obviate the need for unnecessary chemotherapy with its attendant costs, risk of side-effects, danger of drug-resistance, and potential mistreatment of another disease. Insights into genetic and molecular differences between *E. histolytica* and *E. dispar* have allowed for the development of rapid molecular detection assays.[87] Genetic amplification by PCR of genes in which a polymorphism exists between the two species permits differentiation of pathogen from non-pathogen. The greatest difficulty lies in the purification of sufficient quantities of good quality genomic DNA. The source is invariably stool where contaminants and inhibitors of amplification persist. Compounding this is the fact that a significant portion of Entamoeba genomic DNA in stool is encapsulated in cyst form and therefore not easily purifiable. Cyst rupture is not trivial although several labs have suggested apparently successful protocols.[88,89] Sequence analysis of homologous lectin genes (*hgl2* and *dhgl2*) established a simple PCR-RFLP approach that distinguished *E. dispar* from *E. histolytica*.[32]

Using an ELISA approach, patient stool can be rapidly (< 2hrs) screened for antigen.[90] This strategy has proven more sensitive and specific than traditional microscopy and equal to isoenzyme analysis, which is impractical in a diagnostic setting.[28,91] More important, the dissociation of pathogen from non-pathogen allows for identification of patients who actually require treatment. Inherent problems with stool antigen diagnosis of *Entamoeba* exist. Foremost among these are the requirement for fresh or fresh-frozen stool, compounded by the sporadic nature of cyst/trophozoite shedding that requires multiple sampling in order to rule out infection. Stool fixatives such as formalin and PVA appear to preclude both PCR and ELISA diagnosis. Interestingly, antigen ELISA has been used successfully to detect amebic antigen in the amebic liver abscess aspirates.[729] Ultimately, the development of accurate, cheap and fast immunochromatographic strips able to detect multiple enteric pathogens (which present with similar signs and symptoms) in fixed stool samples may revolutionize diagnosis of infectious diarrheal disease.

Summary

Recent developments at the bench have resulted in the redefinition of amebiasis. Traditional E. histolytica is composed of two genetically distinct species, one (*E. histolytica sensu stricto*) which is potentially fatal, and the other (*E. dispar*) a harmless commensal of the gut. Anti-amebic serology can be used to distinguish pathogen (seropositive) from non-pathogen, but more sensitive and specific ELISAs or PCR diagnositic kits may soon be commercially available. Pathogenesis of invasive disease appears to rely on both lectin-like and integrin-like adhesion molecules, as well as a potent mix of cysteine proteases and pore-forming peptides. Single gene knockouts in *E. histolytica* are still required to directly implicate virulence genes in invasion. Liver metastasis provides no evolutionary advantage to the parasite and likely occurs "accidentally" via hematogenous spread (portal circulation), but can result in liver abscesses (Figure 5). Abscesses are readily visualized with ultrasound or CT scan, and can be aspirated transcutaneously. Treatment requires both a lumenal eradication agent (diloxanide furoate) and tissue-active agent (metronidazole). DNA vaccination via mucosal route may provide a useful preventive measure in endemic areas for the future.

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Vaccines for Human Immunodeficiency Virus Type 1

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Abstract

Almost over two decades have passed since the identification of the human immunodeficiency virus type 1 (HIV-1) as the causative agent of AIDS. HIV-1 has proven over many years to be difficult to control. During this period, HIV-1 has been extensively characterized, and a variety of vaccine constructs and strategies have been explored. Despite recent introductions of highly active anti-retroviral therapies (HAART) in the clinics, the compelling need to develop a safe and effective vaccine against HIV-1 remains undiminished. Vaccine approaches have concentrated on eliciting host immune responses against HIV-1 antigens through a variety of immunization methods. Many vaccines are based on strategies designed to mimic natural infection as closely as possible without causing disease, such as using HIV-1 surface proteins or attenuated or killed viruses. Newer vaccine designs have focused on the use of viral vectors or DNA or combination of strategies to elicit immune responses against HIV-1 antigens. The highly variable nature of primary HIV-1 isolates has been one of the major obstacles in the efforts to develop a successful HIV-1 vaccine. This article will present the current state of immunobiology-driven vaccine development for HIV-1, and will describe the obstacles that need to be overcome for the development of a successful HIV-1 to be possible.

Introduction

The human immunodefieieney virus type 1 (HIV-1) has for more than two deeades proven itself to be quite a formidable opponent to humankind. Sinee its first appearance in the late 1970s, the acquired immunodeficieney disease syndrome, or AIDS, eaused by HIV-1, has already resulted in nearly 14 million deaths worldwide. The World Health Organization estimates that in 1998, 33.4 million people are living with HIV-1/AIDS, with more than 95% of the eases oeeurring in the poorest areas of the world.[1] Beeause of this, HIV-1 and its effeets on the human host have extensively been studied, and have especially led to the development of elinieally effective anti-retroviral therapeuties and many tried, but yet unsueeess-ful, vaeeine candidates. Despite the achievements made so far from carefully understanding how the virus works, the global AIDS pandemie eontinues to spread inexorably, making the need for a safe, effective, and more importantly, novel approaches to HIV-1 vaeeine ever more pressing. Even currently available therapeuties eannot contain the virus as drug-resistant progenies are rapidly emerging and as the virus slowly persist latently.[2]

Thanks to the introduction of vaeeinology by an insightful physician-scientist Edward Jenner more than 200 years ago,[3] history has now repeatedly shown that vaccines and the benefits they conferred are the most effective and cost-effeetive means of eontrolling infectious diseases worldwide. Vaccination has resulted in the eradication of smallpox and the control of polio, yellow fever, and measles. Vaeeines are the most dramatic examples of the salutary eonsequenees of the scientifie approaeh to human health and disease. Although HIV-1/AIDS transmission is influeneed by social dynamies that ean partly be preventable by safe sex educational programs and can be temporarily eontrolled with antiretroviral therapeutics for those who can afford them, a vaecine against HIV-1 is required in order to make a significant impaet on HIV-1 transmission and disease worldwide. This need is partieularly evident in areas of the world where prevalenee of HIV-1 and AIDS approaehes or exceeds 30% (espeeially in sub-Saharan Afriea and Southeast Asia), areas where aeeess to pharmacologieal treatments and healtheare is limited. The following will deseribe the current status in HIV-1 vaccine development.

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HIV-1 Vaccines - The Ideal Characteristics of an HIV-1 Vaccine

There are two main kinds of vaccines: (i) preventative and (ii) therapeutic. Most of the vaccines (e.g. influenza, hepatitis B, measles, etc.) that we are familiar with are preventative vaccines. These vaccines aim to elicit a potent and long-lived immune response in the healthy non-infected recipient to provide some level of immune protection against the potential of future infections. Therapeutic vaccines are given to individuals already infected with the pathogen, with the goal of boosting and enhancing the immune response in the hopes of lowering the virus load, and thus disease burden. This review will concentrate mostly on preventative HIV-1 vaccines, with mention to therapeutic vaccines when relevant.

In any attempt to develop a human vaccine against infectious disease agents, one must know what to strive for. Many lessons have been learned from past experiences in developing vaccines that were successful against viral infectious agents such as the smallpox and polio viruses. Additionally, by understanding the nature of HIV-1 and its effects on the host, one can take a more rationale approach to vaccine design against the virus. Theoretically, an ideal AIDS vaccine should have the following properties:

1. **Effective prevention of HIV-1 transmission** by the mucosal and parenteral routes;
2. **Ability to induce long-lived protective immune responses** (antibodies, cytotoxic T cells, helper T cells) against diverse primary virus isolates, obviating the need for many strain-specific vaccines;
3. **Excellent safety profile,** with minimal risk of adverse reactions;
4. **Practicality:** high stability, ease of production and administration, and low cost, facilitating widespread immunization campaigns in the developing countries.

However, there has yet been an HIV-1 vaccine developed to date that satisfies the criteria of complete protection from infection, or sterile immunity, as described above. This has led many AIDS researchers to pursue the next best. In fact, a handful of candidate vaccines, not expected to offer perfect protection or complete viral eradication, are currently in phase III clinical trials. Such partially effective vaccines could in theory be beneficial to vaccine recipients by preventing or moderating disease, rather than the infection itself. The alternative goal for the AIDS vaccine would then be to induce immune responses that can control HIV-1 replication, potentially resulting in a prolonged and maybe indefinite asymptomatic period, a state similar to those of long-term survivors (LTS) or long-term nonprogressors (LTNP).[4, 5] In support of this, vaccine studies in nonhuman primates have occasionally demonstrated either lower viral loads or prolonged survival of immunized animals, even in the absence of sterile immunity.[6] Vaccines could thus be designed to abort infections by clearing incoming viruses and infected cells, or could control infection and reduce infectivity at some level without the manifestations of clinical disease. In fact, it has been shown that lowering the initial viral setpoint can lead to better clinical outcomes for the individual.[7] This setpoint is clinically defined to be the virus load at 6 months post-HIV-1 infection. Conceptually, this represents the stable and equilibrated level of virus in response to acute viremia that depends on factors such as immunologic response, host cell susceptibility and viral fitness, to name a few.

In a population setting with high rates of infection, mathematical modelings using data from epidemiological studies have indicated that many cases of HIV-1 infection and its associated mortality could be prevented by immunogens with efficacy of 50% or less and a five year protection duration.[8] A reduction of infectivity of those vaccinated could have an impact upon the population at large, providing some level of 'herd immunity.'[9] This could potentially be achieved with a less than perfect HIV-1 vaccine. With this re-evaluated goal in mind, the development of a beneficial HIV-1 vaccine could theoretically be soon achieved.

Barriers to the Development of an Effective HIV-1 Vaccine

There have been a number of obstacles to the development of an effective AIDS vaccine. First, the extreme sequence variability of the HIV-1 virus, as a result of mutations introduced by the error-prone reverse transcriptase and recombinations between different viral strains,[10] poses a considerable barrier to vaccine development. *In vivo*, it is known that HIV-1 replicates rapidly, producing up to 10^{10} new virions per day, which facilitates the rapid generation of sequence variants.[11] The highly mutable nature of the HIV-1 is especially evident when selection pressures are imposed by either the immune system or by anti-viral pharmacological drugs. The result is a tremendous sequence variation in the envelope protein between various HIV-1 clades and strains. With some exceptions, most neutralizing antibodies and cytotoxic T lymphocytes (CTLs) identified against HIV-1 envelope have been virus strain-specific and would not be effective against other viral variants.[12, 13] Thus, the goal of vaccine design has been to induce more broadly reactive immune responses against relatively conserved viral epitopes, which are few in numbers and tend to be only moderately immunogenic at best.

Second, there has been a lack of information regarding what type of immune response is required to provide beneficial protective immunity against HIV-1. This problem has been further compounded by the state of eventual immunodeficiency as a result from the HIV-1 infection itself, which makes it difficult to properly assess true protective correlates. Over the past decade, it has becoming clear that susceptibility to HIV-1 infection and disease is quite variable. In fact, among susceptible individuals exposed to HIV-1 infection, not all become infected, and among those that are infected, not all develop disease. Clinical studies in these rare patients, classified as either exposed and uninfected (EU), infected but long-term nonprogressors (LTNP) or, infected but long-term survivors (LTS), have provided some important clues as to what potential mechanisms are responsible for the protection. Lessons learned from those with high degree of documented HIV-1 exposure, in whom persistent seronegativity is more likely to indicate genuine resistance to infection (e.g. EUs), are especially important in providing key insights into what constitutes natural

immunity, which will provide better rationales in vaccine designs.[14-17]

Third, HIV-1 as a retrovirus integrates into the host genome and in <1% of the infected cells, they remain latent. Latently-infected cells express few HIV-1 structural proteins, and are thus not as likely to be eliminated by the host cellular and humoral immunity. This is an issue to consider particularly with regards to therapeutic HIV-1 vaccines in infected individuals, already harbouring latent virus, as strategies are needed to improve immune recognition of the latently-infected host cells. Also, the virus persists for a long time, especially in resting memory $CD4^+$ T cells,[18, 19] and could easily be reactivated even after prolonged suppression by potent antiretroviral therapy.[20-22] Furthermore, the virus is continually undergoing rapid and persistent replication even in patients with sustained suppression of plasma HIV-1 RNA to undetectable levels (<50 copies/mL) as a result of prolonged treatment with HAART.[23] Because of the viral persistence, it has been estimated that it would take over 60 years to completely eradicate the latent reservoir under the best current anti-HIV-1 therapy.[24] Fortunately, Chun *et al.* has recently shown that IL-2 administration, with the help of HAART, can induce HIV-1 expression from latently infected cells, and lead to their substantial reduction by the immune system.[25] However, when patients were taken off HAART despite the use of IL-2, all the IL-2-treated patients had virus return quickly.[2]

Fourth, HIV-1 can be transmitted by both cell-free and cell-associated viral particles; however, most non-human primate challenge experiments evaluate vaccines using only cell-free viruses. Incoming virally-infected cells are thought to be relatively resistant to neutralizing antibodies and will not be recognized by viral-specific host CTL responses. Thus any vaccine efforts should consider strategies to better immunologically intercept these infectious cells.

Fifth, HIV-1 is predominantly transmitted by the mucosal route, unless the viral exposure is intravenous from drug use or from tainted blood products which accounts only for a small percentage of all transmissions. Unfortunately, very little is known about what happens at the mucosa during HIV-1 infection; even less information is known about what constitutes protective mucosal immunity. A number of strategies to induce effective immunity at the mucosal site is now beginning to accumulate.[26, 27]

Sixth, the lack of exact animal model for vaccine testing poses another problem. Efficacy of vaccine candidates are not readily studied in humans since viral challenge is not feasible and so would require huge trials in high-risk volunteers. In the commonly used non-human primate models for HIV-1, HIV-1 does not infect macaques and does not cause AIDS-like disease processes in chimpanzees,[28, 29] resulting in unresolved questions regarding their direct application to humans.

Finally, there are multiple ethical and practical matters that need to be dealt with especially with issues concerning vaccine testing. There are tremendous financial disincentives for companies to invest in a vaccine that caters to third-world nations. As a consequence, the majority of vaccines developed to date have been against the North American clade B virus. Unfortunately, >95% of all HIV-1/AIDS cases are in the developing world. There are also ethical dilemmas associated with the inclusion of placebo control groups in clinical vaccine trials. With requisite counseling and condom use promotion, it would be difficult to setup a study with sufficient statistical power. It is therefore clear that the barriers to the development of an effective HIV-1 vaccine are tremendous.

Current Vaccine Approaches

A preventative HIV-1 vaccine can either elicit an antibody, cellular immunity or both, depending on the type of vaccine constructs used. **Antibody immunity** is the only immune response shown previously to induce protection in other vaccines for human use such as measles and hepatitis B. No one has yet been able to directly correlate **cellular immunity** (both $CD8^+$ CTLs and $CD4^+$ helper responses) to protection in previous vaccines. The advantage of antibody induction is that it can clear and neutralize HIV-1 immediately upon exposure; however, it has repeatedly shown that it is difficult to design vaccine immunogens that can effectively induce such broadly HIV-1 neutralizing antibodies. From a recent exciting finding, LaCasse *et al.* were able to generate extensively broadly neutralizing antibodies in mice transgenic for the human CD4 HIV-1 receptor and CCR5 chemokine co-receptor using fixed HIV-1/target cell fusion complexes as immunogens.[30] They suggested that previously undiscovered HIV-1 epitopes with greater immunogenicity might be exposed or created as the virus begins to fuse with host target cell membranes. It remains to be seen what these epitopes are, and if such fusion complexes could elicit similar responses in primate models and provide protection against viral challenges. The cellular response, on the other hand, is particularly helpful in clearing HIV-1-infected cells; however, it takes a number of days for such cellular immunity to develop, even with a vaccine. Therefore, a fast-replication agent like the HIV (producing 10^{10} viral particles/day with a half-life of 6 hours) may be able to over-ride a rapidly mounting cellular immune response; thus, cellular immunity alone may not be enough to eradicate and control the virus.[31] A vaccine that can induce both responses might be the most useful. Work by Dittmer and Hasenkrug, although in the retroviral Friend leukemia virus mouse model, has elegantly shown that immune responses involving the combination of B cells, $CD4^+$ T cells and $CD8^+$ T cells, are required to provide protection against retroviral infections, and that any of these immune cell subsets cannot on their own provide similar levels of protection.[32] Thus, their findings may have important implications for vaccine against the retrovirus HIV-1.

In the following section, the current approaches to HIV-1 vaccine will be described, with special emphasis on the strategies that appear to be more promising, including those presently in clinical trials. Table 1 summarizes the different vaccine strategies, highlighting their benefits and limitations.

(a) Live, Attenuated HIV-1

A live virus that is genetically altered to attenuate its pathogenic potential offers the best alternative to its natural wildtype counterpart for many reasons: presentation of viral antigens in their

Table 1
Current Strategies in HIV-1 Vaccine Development

	Tested in non-human primates	Tested in humans	$CD4^+$ T cell proliferative response	$CD8^+$ CTL repsonse	Neutralizing antibodies v.s lab-adapted strains	Neutralizing antibodies vs. primary isolates
Live, attenuated HIV	+	–	+	+	+	–
Inactivated virus	+	+	+	rare	+	–
Recombinant subunit vaccines (proteins/peptides)	+	+	+	rare	+	–
Live vector-based vaccines	+	+	+	+	+/–	–
DNA vaccines	+	+	+	+	+	–
Combination vaccine strategies	+	+	+	+	+	+

natural forms (e.g. with regards to conformation, glycosylation and oligomerization); prolonged antigen delivery for a longer lived immunity; induction of strong $CD8^+$ CTL responses. However, there are extreme safety concerns associated with its use, especially with regard to its ability to recombine with wild-type virus generating infectious recombinant progeny. Despite this potential risk, there was some early excitement with this approach as vaccination of macaques with a *nef*-deleted live SIV resulted in one of the most effective vaccination approaches tested to date.[33] This approach provided protection against a pathogenic strain of SIV for as long as 3 years after a single inoculation of the vaccine. The enthusiasm was further fueled by the identification of 'resistant' HIV-1-tainted-blood recipients who were infected with a *nef*-deleted virus.[34] However since then, complete regeneration of the deleted *nef* virus to its wildtype state has been observed in infected macaques,[35] and gene-deleted variants of SIV have proven to be pathogenic to neonate[36] and adult macaques[37] alike, given enough time. There have also been subsequent reports describing progressive immunologic damage and increasing levels of detectable viral loads in the human recipients of *nef*-deleted virus.[38] In an effort to make live HIV-1 vaccine a viable option, others have made multiple gene deletions to cause further attenuation[39] or incorporate a suicide gene to confer susceptibility of the virus to conventional antiviral agents.[40] Unfortunately, it is relatively clear that the protective efficacy of these vaccines decline with each additional level of attenuation.[3441]

(b) Inactivated HIV-1

In spite of the fears that incomplete inactivation of the virus might lead to inadvertent infections of individuals being vaccinated, this strategy early on in the AIDS epidemic have also garnered considerable interest. Studies showed that macaques receiving inactivated SIV were readily protected against homologous pathogenic SIV challenges.[42, 43] However, enthusiasm for this approach quickly waned when subsequent findings suggested that the protective immunity responsible was not virus-specific, but had to do with host cellular proteins.[44] The potential beneficial role of these non-viral host proteins (especially the major histocompatibility complex, MHC, proteins) in providing protection against HIV-1 has been investigated by a number of laboratories, including our own, and their implication in HIV-1 vaccine has recently been reviewed.[45] Even if inactivated virus could be used as a vaccine, there is concern that the inactivation process might disrupt immunogenic viral antigens. Thus, efforts have been made to express in cell lines viral proteins in their native conformation that can self-assemble into non-infectious replication-incompetent viral particles (also known as pseudovirions or virus-like particles). One of these approaches currently in clinical trials is the yeast Ty transposon p1 protein, fused with HIV-1 gag proteins, which can assemble into 50-nm virus-like particles and elicit broadly directed immune responses. Currently, a gp120-depleted, inactivated HIV-1 in incomplete Freund's adjuvant (called Remune) is being used as a therapeutic vaccine in HIV-1-infected individuals, and have resulted in the augmentation of HIV-specific functional immunity.[46] HIV-specific memory immune responses can also be stimulated by Remune in subjects with chronic HIV infection; however, whether the induction of such responses is associated with virologic control remains to be seen.[47]

(c) Recombinant HIV-1 Subunit Vaccines (proteins/peptides)

Relevant purified viral proteins or peptides recombinantly produced (especially those focusing on the viral envelope protein) provide a much safer alternative to whole virus-based vaccines. However, their greatest limitation resides in their ineffective induction of both protective HIV-1 specific CTLs and neutralizing antibodies against patient HIV-1 isolates. These failures can be attributed among others to poor mimicry of native HIV-1 proteins in terms of conformation, glycosylation and oligomerization, and inefficient access to MHC class I antigen presentation pathway for $CD8^+$ T cells induction. In most cases, adjuvants are required to improve immunogenicity. Numerous formulated adjuvants have been used in conjunction with the subunit vaccines which include alum, incomplete Freund's adjuvant, MF59 (stable emulsion consisting of metabolizable oil and surfactants), MPL (monophosphoryl lipid A), polyphosphazene, virosomes (viral proteins in lipid vesicles), MTP-PE (muramyl tripeptide from mycobacterial cell wall), and soluble QS-21 or particulate ISCOMs (immune stimulating complexes or saponins from the Quillaja soapbark tree).[48,49]

For the most part, vaccination with recombinant subunit vaccines has been unsuccessful in providing protection in animal models, with the exception of protection after immunization with a recombinant gp120 protein in chimpanzees.[50] However, the challenge virus in the study was lab-adapted and known to replicate relatively poorly in non-human primates.

In humans, extensive testing has yielded unspectacular results: that is, relatively poor neutralizing activity against primary viruses,[51] poor CTL induction, and breakthrough infections in phase II studies.[52] Despite these outcomes, phase III trials,[553] the first of its kind for an HIV/AIDS vaccine, are currently underway in North America and Thailand to test a bivalent version of this gp120 protein vaccine (AIDSVAX from VaxGen). The AIDSVAX is made up of the envelope proteins from two different clades of the HIV-1 (clade B, found mostly in North America and clade E, found mostly in Asia).[54] The bivalent (B/E) vaccine has recently been shown to induce antibodies that can neutralize primary HIV-1 isolates *in vitro*.[55]

While results from the phase III human trial are pending and may yield some interesting clinical data, efforts should continue at ameliorating this particular strategy. To this end, recombinant envelope glycoprotein immunogen should be based on sequences derived from patient isolates, expressed as oligomers, and be presented to the immune system as similar in their tertiary conformation as the wildtype virus. The changes that take place in the envelope protein upon viral binding to host CD4 and chemokine receptors should also be considered. Synthetic peptide vaccines, used with adjuvants, offer the benefit of targeting multiple specific epitopes in conserved regions of the virus as a strategy for overcoming the problem of HIV-1 sequence diversity. Much work has focused on antigenically mimicking the V3 loop, for example; but, neutralization determinants exposed in the V3 loop of lab-adapted HIV-1 are not present in patient isolates.[56, 57] However, the V3 loop may be important in gp120-CD4-chemokine receptor interactions, and thus work on this continues. Early human trials have found octameric V3 peptides bound to a heptalysyl core with alum,[58] or conjugation to *M. tuberculosis* purified protein derivative (PPD), to be immunogenic. But either of the recombinant protein or peptide strategies probably cannot stand on their own, and may work best in combination with other vaccine strategies.

(d) Recombinant Live Vector-Based Vaccines for HIV-1

Other viruses or bacteria can be recombinantly engineered to express HIV-1 proteins for use as live vaccines capable of eliciting immunity of the magnitude and duration similar to a natural infection, except without the pathogenic consequences. However, similar to the problems with gene therapy, this approach is constrained by the size of the insert gene, the *in vivo* replication capacity of the vector, immunity to the vector agent, genetic instability and the potential pathogenicity of the recombinant organisms themselves, especially in immunocompromised individuals. The most intensely studied vaccine vectors thus far are the avipox and vaccinia viruses (the same virus that helped eliminate worldwide smallpox infections). Initial use of vaccinia expressing SIV antigens elicited both impressive humoral and cellular responses in macaques[59, 60] and chimpanzees,[61] and provided protection against infectious challenges in some cases. However, because of concerns regarding its safety, as it could cause life-threatening disseminated disease in immunocompromised individuals, more recent efforts have focused on highly attenuated forms of these viruses. One is the modified vaccinia ankara (MVA) virus, an *in vitro* multiply-passaged vaccinia strain with a number of genes deleted to decrease its pathogenicity (e.g. NYVAC). A NYVAC vector expressing HIV-1 antigens induced cross-protection against HIV-2 challenge in rhesus macaques,[62] and provided some protection especially against mucosal challenges.[63] Another related virus, thought to be safer, is the avian pox virus, such as the canarypox. This virus does not complete their entire replication cycle in humans, but initiates synthesis of foreign proteins and elicits immune response (e.g. ALVAC). Canarypox vectors have been tested already in more than 300 uninfected volunteers, demonstrating their *in vivo* B and T cell priming capacity, albeit variable in nature with limited durability.[64, 65]

Other viruses are also being evaluated as candidate vaccine vectors in preclinical studies. Adenoviral vectors have the attractive feature of inducing both mucosal and systemic immune responses after oral administration, but to be effective in humans, this approach would require overcoming pre-existing or acquired immunity to the vector itself. Such a vector expressing SIV proteins can induce SIV-specific humoral, cellular and mucosal responses, resulting in decreased viral loads after vaginal viral challenges.[66] Live attenuated poliovirus can also induce mucosal and systemic immunity.[67-69] The single-stranded RNA alpha viruses which includes the Semliki forest virus[70, 71] and the Venezuelan equine encephalitis (VEE) virus[72-74] have demonstrated impressive pre-clinical results. These alpha virus vectors can stimulate a comprehensive humoral and cellular immune response, including those at the mucosal surfaces. Recombinant bacteria have also been tried as HIV-1 vaccine vectors. Bacille Calmette Guerin (BCG) from the avirulent *Mycobacterium boris* has been successful in providing safe and long-lasting protection,[75] even when given to newborns, and can induce CTL immunity in rhesus monkeys.[76] Enteric bacteria such as salmonella have some generated interest because of their ability to specifically elicit mucosal responses; however, convincing results for its efficacy, in primates, including humans, are currently lacking.[77] However, these recombinant live viral vector vaccines are all limited by the compromised immunogenicity associated with the degree of attenuation made to reduce their in vivo replication or to directly reduce their virulence.

(e) DNA Vaccines for HIV-1

DNA vaccination has, over the past few years, proven to be very effective at inducing both antibody and infection-independent $CD8^+$ CTL responses to the expressed antigens of a range of infectious agents including HIV-1 and SIV, and in many cases providing good protection against pathogenic challenges (reviewed in 78-81). DNA vaccines mimic the effects of live attenuated vaccines in their ability to induce CTL responses, which may be advantageous compared with the conventional protein-based vaccines while mitigating some of the safety concerns associated with live vaccines. The DNA vaccine has a number of qualities that make it an attractive approach:

(1) it is an elegant technology that is effective at inducing immune responses; (2) it is easy and cheap to manufacture – any viral gene of interest can easily be cloned into a DNA vector for immunization; and (3) it is relatively stable in warm climates - do not require sophisticated storage set-up for distribution, obviating the need for a 'cold chain' which is often a significant limitation for vaccines used in developing nations.

The most effective means of delivery of the plasmid DNA encoding the foreign antigen have been either intramuscular via needle injection or intradermal with the aid of a 'genegun.' Professional antigen presenting cells (APCs) especially dendritic cells (DCs) take up the DNA, express the protein encoded in the DNA, and process the nascent proteins into MHC-binding peptides. This triggers strong antibody and CTL immunity, and in several cases protective immunity against pathogenic challenges. The efficient induction of a broad-based and sustained immune response is partly due to the bacterial plasmid DNA itself having immunostimulatory properties due to their unmethylated cytidine phosphate guanosine (CpG) dinucleotide motifs.[82,83] Another reason may have to do with the type of APCs transfected. It has been conclusively shown that bone-marrow derived APCs, and not somatic cells (such as myocytes or skin keratinocytes) play a key role in the direct induction of the immune response following DNA vaccination.[84-86] Furthermore, it has been demonstrated that the predominant contribution to priming immune responses after DNA vaccination involves a small number of directly transfected dendritic cells.[87-90] However, because muscle cells or keratinocytes constitute the predominant cell transfected after DNA inoculation via muscle or skin injections, respectively, these somatic cells could serve as antigen reservoirs and 'protein factories'. Upon DNA vaccination, it has been shown that there is a general activation and migration of large numbers of untransfected DCs, which could present antigens via alternative mechanisms such as cross-priming or 'antigen transfer'. Secreted or exogenous antigens generated by DNA-transfected somatic cells (myocytes or keratinocytes) could be taken up by professional APCs to prime for T cell responses, as evidenced by the effective T cell priming in antigen-expressing myoblasts transfer studies.[91,92] More recently, there is evidence suggesting that the bulk of the immune response from intramuscular DNA vaccination is dependent on expression of antigen by non-lymphoid tissues and antigen transfer to bone marrow-derived APCs from studies using a transactivating plasmid system and bone marrow chimeras.[93] It is clear that muscle cells can take up the DNA and express the protein; however, because of their inherent poor expression of co-stimulatory molecules (such as the B7 molecules: CD80 and CD86) which are required for optimized T cell priming, muscle cells alone are not efficient at priming immune responses following DNA vaccination.[94] In fact removing the muscle 10 minutes immediately after DNA injection does not alter subsequent immune responses.[95] This suggests that transfection of muscle cells may even be unnecessary as the injected plasmid DNA can reach the lymphatic or circulatory system and transfect cells at these sites. The skin, on the other hand, contains keratinocytes and Langerhans' cells that are transfected upon DNA injection that can prime for CTL responses.[96,97] Moreover, skin keratinocytes are required for their continued production of antigens to augment the immune response.[95,98] For a summary of the current understanding of how DNA vaccination works, see Figure 1.

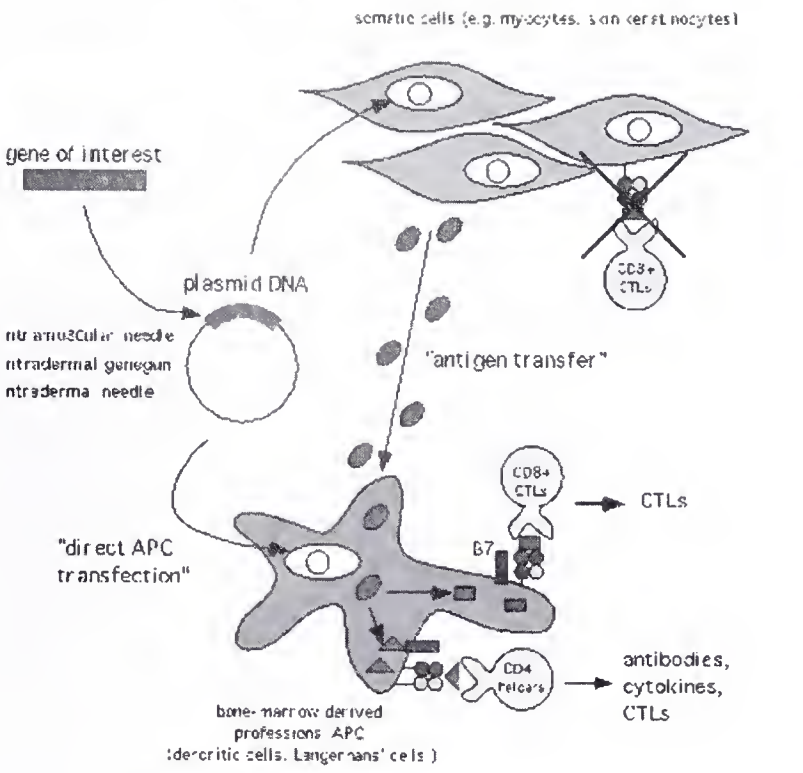

Figure 1. Schematic diagram of the current thinking of the mechanisms for DNA or nucleic acid immunization. Direct priming by transfected somatic cells (e.g. muscles) has been shown not to occur. It has been shown that bone-marrow derived professional antigen presenting cells (APCs) such as dendritic cells are responsible for priming immune responses. These cells express co-stimulatory molecules such as the B7 molecules (CD80, CD86) and CD40. The professional APCs either acquire antigens through "antigen transfer" from non-hematopoietic cells acting as an antigen factory, or through "direct APC transfection" and antigen is synthesized by the APCs themselves for presentation to naive T cells.

There have now been many reports on the use of DNA vaccine to deliver HIV-1 antigens in both non-human primates[99-104] and humans,[105-108] involving various schedules and routes of immunization. These studies have shown that DNA vaccines generate SIV- and HIV-1-specific CTL, helper T cells, antibodies, and in some cases, mucosal immune responses when the DNA was administered mucosally.[109] However, in macaques, most DNA vaccines have been particularly unsuccessful at protecting against infection with either SIV or SHIV strains of high replication potential, although lowering of viral loads has been observed.[100] It should be noted that Boyer *et al.* have shown that with a multivalent HIV-1 DNA vaccine, protection can be achieved in chimpanzees albeit against a non-pathogenic HIV-1 strain.[103]

(f) Combined Vaccine Approach Against HIV-1

Investigators have combined different vaccine strategies in the hopes of optimizing the *in vivo* responses of any single vaccine. "Prime-boost strategies" as they are called, often involve a first vaccination that can generate a cellular immune response such as a viral-vector based vaccine or DNA vaccine, followed by vaccinations that are particularly effective at inducing strong T-helper responses and high-titre antibody responses such as a subunit protein.[110] These types of prime-boost vaccination protocols have yielded quite encouraging responses in macaques and chimpanzees,[111] and more recently in humans.[112] For example, recombinant vaccinia virus expressing gp160, followed by a boost with recombinant gp160, protected macaques

against intravenous challenge with homologous SIV strain,[113] but not with heterogeneous viruses.[114] Protection against HIV-2 challenge was also observed with prime-boost protocols involving ALVAC and protein vaccines.[77] Combinations of vaccinia and DNA encoding viral epitopes have been effective at inducing strong CTL responses.[115] Potent immunity and protection were achieved when DNA was combined with a protein boost.[116] Subsequently, Robinson and colleagues have found that amongst the different combinations of vaccines tried, the most promising vaccine regime was intradermal DNA priming followed by boosting with recombinant fowl pox virus.[117] This particular prime-boost approach, which corroborates results from an earlier study,[118] was successful at containing infection upon multiple challenges including one that involved a pathogenic strain of virus. Both groups attributed the protection to T-cell mediated mechanisms, and not to neutralizing antibody responses. Adenovirus vectors were also used in combination vaccine regimes as disease progression was slowed in a few animals that were given SIV envelope-expressing adenovirus and protein gp120.[119] A number of these combination vaccines have already been tested in humans,[65, 120-124] with the earlier combined vaccine work by the late Clements-Mann spearheading this approach.[125] A handful have already entered into various phases of clinical trials, with human efficacy results pending.

Redefining the "Ideal" HIV-1/AIDS Vaccine

There is no doubt that numerous strategies have already been attempted in pre-clinical studies involving virus challenge models using non-human primates. Repeatedly, vaccine candidates that have shown good protection against viruses that replicate relatively poorly more often cannot prevent infection the same way against viruses that replicate to higher levels. This is important considering HIV-1 replicates at high levels in humans. It is unfortunate that there have only been very few examples of vaccine candidate tested that has resulted in sterilizing immunity against highly pathogenic challenges, the gold standard of any vaccine endeavour. One of these strategies involved the use of gene-deleted live attenuated retroviruses as described above, which have their inherent risks for symptomatic disease. The other came from studies using whole inactivated virus, with protection attributed more to immunity raised against host cell MHC proteins.[44, 126-131] Thus, it is seemingly easier to reduce post-challenge viral loads and prevent or delay clinical disease progression than preventing the initial infection altogether. This could be achieved in the absence of sterilizing immunity, prompting a re-evaluation of 'the gold standard.' A more realistic goal for an HIV-1/AIDS vaccine would be to (1) dampen viral replication during primary infection, (2) to lower the steady-state of viral replication during chronic infection, thus reducing the infectivity of those infected, and (3) to slow the progression to clinical disease. In fact, these observations have already been made in many nonhuman primate vaccine trials, in the absence of complete sterilizing immunity.[132] This is why a number of vaccine approaches have already earned their way to testings in human clinical settings, with a few currently entered into large-scale phase III trials (such as Vaxgen's recombinant gp120 vaccine (AIDSVAX), currently being tested in Thailand and in the U.S.) even though they may be less than 'perfect'. It has been rationalized by many that even a partially effective HIV-1 vaccine could be beneficial to both high-risk individuals and to the population at large, potentially prolonging survival for those who subsequently get infected and diminishing the likelihood of virus spread to others uninfected.[133]

Conclusion

There are thus far numerous different vaccine strategies being tested against HIV-1, with less than a handful currently being tried in large-scale clinical trials. Results from these human trials will not be available for another few years to determine their efficacy at preventing viral infection and at controlling viral replication. However, there are a number of HIV-1 vaccine alternatives in the pre-clinical testing stage that show promising results. Efforts should continue in developing novel strategies against the virus, especially ones that can circumvent the problem of viral antigenic variability. There are some reasons to be hopeful about the prospects for a successful HIV-1 vaccine. First, there are a number of neutralizing antibodies against relatively conserved B cell epitope on the HIV-1 envelope protein that have been found to be effective at inhibiting viral replication. Work in revealing more of these HIV-1 epitopes will only help the vaccine effort. Second, there is strong evidence in the non-human primate models suggesting that sterilizing immunity can be achieved by the use of host cell proteins such as class I or class II MHC as immunogens.[45] Continued work in this alternative approach to HIV-1 vaccine is warranted to determine its applicability in humans. Third, there are now well-established group of HIV-1-exposed, but uninfected individuals that may possess protective immunity. Fourth, there are also now defined groups of HIV-1-infected, but asymptomatic, long-term non-progressors, with stable CD4 counts for more than 10 years post-infection. This may suggest that these individuals may have some level of immunological control of the virus. And last, there are new promising vaccine strategies such as plasmid DNA immunization that can offer the potential to induce strong CTL and antibody responses without infection, especially in combination with other vaccine approaches. Better understanding of what constitutes as true immune correlate of protection from HIV-1 will aid in a more rationale approach to vaccine design. Ultimately, it is believed that vaccines, even if it is partially effective, can provide significant benefits to the individual and to the population as a whole against this formidable pathogen. However, it is clear that more work needs to be done for the goal of developing a successful HIV-1/AIDS vaccine to become a reality.

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In the Literature

Yvonne Chan, M.Sc. (OT2) and Stephen C. Juvet, B.Sc. (OT2)

This section of News and Views will present updates of recent advances in the medical and scientific literature.

Perioperative Oxygen Therapy Can Reduce the Risk of Surgical Wound Infection

The major mechanism of bacterial destruction following surgical wound infection is oxidative killing, a proeess whieh is known to be dependent upon loeal tissue eoneentrations of oxygen (O_2). In a well-perfused tissue, the local oxygen tension can be improved with inhaled O_2. Greif and eolleagues hypothesized that perioperative inhaled O_2 might reduee the ineidenee of surgieal wound infeetions. Five hundred patients scheduled to undergo eoloreetal resection were randomly assigned to reeeive either 30% O_2 or 80% O2 during surgery and for two hours following. All of the patients reeeived the same anaesthetic and prophylaetie antibiotics. The outcome measure was the presenee or absence of a culture-positive exudate in the surgieal site at any time postoperatively. Wounds were evaluated daily in a double-blind fashion until the patient was diseharged, and 2 weeks later at a follow-up elinie visit. Of the 250 patients who reeeived 80% O_2, 13 (5.2%) had surgical wound infections while 28 (11.2%) of the 250 patients who received 30% O_2 had surgieal wound infeetions, a significant difference. The authors eonelude that perioperative O2 administration is a simple and inexpensive method to prevent surgieal wound infections.

(Greif R, Akca O, Horn E-P, et al. NEJM. (2000). 342: 161-7)

Correlation Between Neuropathology and Clinical Dementia in Alzheimer's Disease

An area of eontroversy in the neuroseiences has been the apparent laek of correlation between the neuropathologieal lesions found in the brains of patients with Alzheimer's disease (AD) and the degree of elinical dementia present in these patients. Two distinet types of pathologie lesions have been deseribed in AD: plaques containing amyloid α-peptide (A+) and neurofibrillary tangles (NFTs) containing the cytoskeletal protein tau. Näslund and co-workers hypothesized that the formation of either or both of these lesions should correlate with the clinical signs of dementia. They eondueted a postmortem cross-seetional study of 79 nursing home residents with elinieal dementia rating (CDR) seores (assigned on the basis of antemortem reeords) ranging from 0.0 (no dementia) to 5.0 (severe dementia). Patients with neurodegenerative diseases other than AD were exeluded. Brain tissue samples from the frontal, temporal, entorhinal, parietal, and primary visual cortiees were prepared, exeluding white matter. The brain tissue samples were homogenized and an A+ supernatant was extracted. The A+ in eaeh sample was quantitated using ELISA tests and immunoblotting with monoelonal antibodies direeted at A+x-40 and A+x-42, two alternate forms of A+ found in brains afflieted with AD. Tau was quantitated immunohistoehemieally. The quantities of both A+ peptides were signifieantly correlated with CDR seore for all of the brain regions except the primary visual eortex. They were also strongly eorrelated with the traditional neuropathologie diagnostie eriteria for AD. Sinee NFTs ean be found in the brains of normal aged individuals and only appear late in the frontal cortex in AD, only this region was analyzed for tau. The results showed that in early dementia (CDR 1.0-2.0), A+x-40 and A+-x42 levels peak first, followed by NFT deposition, and ultimately, disease progression. The authors speculate that many of the histologieal eharaeteristics of AD brains only appear late in the disease, and that in early eases, there is a significant quantity of soluble and/or submieroseopie A+ in the brain tissue. Given that NFTs are known to oeeur in the absenee of amyloid plaques, this notion might salvage the existing hypothesis that NFTs form as a neurotoxic response to the presenee of the plaques. These results elearly demonstrate that there is a strong eorrelation between the extent of the neuropathology of AD and its elinieal manifestations, partieularly in the early stages. Furthermore, they are eonsistent with the hypothesis that NFTs are formed in response to the presenee of A+.

(Näslund J, Haroutunian V, Mohs R, et al. JAMA. (2000). 283:1571)

Should Paramedics Treat at the Scene or Should They "Scoop and Run"?

Prehospital management of eritieally injured patients by Emergency Medical Services (EMS) personnel is a eontroversial issue. A previous retrospective study by Demetriades *et al.* (*Arch Surg.* 1996; 131: 133-138) demonstrated a higher mortality rate in trauma patients transported by EMS compared to those privately transported. The outeome differences were not explained by demographics, patterns of injury, or severity of injury. These findings led Cornwell and colleagues to hypothesize that there was a transport time difference between the two groups of patients, whieh resulted in different outeomes. The authors conducted a prospective cohort-matehed observation study examining various outcome measures sueh as time from injury to hospital, mortality, morbidity, and length of hospital stay, in a group of EMS-transported patients versus those who were non-EMS transported. A total of 103 patients at a level I trauma centre were enrolled in the study, including 38 non-EMS patients, 38 EMS matched, and 27 random EMS patients as controls. Matehing eharaeteristics consisted of age, Injury Severity Score (ISS), mechanism of injury, head Abbreviated Injury Score (AIS), and presenee of hypotension. Results showed that there was no significant difference between the seriously injured non-EMS and matched EMS group in terms of demographic data, mortality, length of hospital stay, and complications. Furthermore, there was no time differenee among the entire sample of the two groups; however, in more eritically injured patients (ISS ≥13), the non-EMS patients arrived at the hospital in significantly less time than the EMS group (an average of 15 minutes vs. 28 minutes, $p<0.05$). A regression analysis demonstrated that ISS scores strongly correlated with outcome, and while transport time did not reach statistieal significance, there was a trend toward better outcomes with shorter arrival intervals. The longer transport time in the more critically injured group may have resulted from attempts to stabilize the patients by giving intravenous fluids and with immobilization to prevent spinal injury at the seene. Unfortunately, prolonged attempts at resuscitation and stabilization in the field may simply be inadequate in some seriously injured patients; however, a "scoop and run" approach may also neglect important issues, such as a compromised airway. In summary, this study suggests that a scene-based EMS response may be more appropriate than an across-the-board protocol.
(Cornwell EE, Belzberg H, Hennigan K, et al. Arch Surg. (2000) 135: 315-319)

Antihypertensive Therapy and the Risk of Type 2 Diabetes Mellitus

Type 2 diabetes mellitus affects more than 7 million Americans and leads to more than 300,000 deaths annually. More importantly, antihypertensive medications are widely prescribed and used by more than 20 million adults in the United States alone. The use of these drugs raises a concern, as a number of previous studies have found that thiazide diuretics and beta-blockers may promote glucose intoleranee, resulting in the development of type 2 diabetes mellitus. However, those studies were compromised by small numbers of patients, relatively short follow-up periods, a lack of comparison groups, and inadequate selection criteria. Due to the limited generalizability of these studies, the risk of developing type 2 diabetes mellitus as a consequence of antihypertensive therapy remains a controversial subject. Gress and eolleagues have overcome these limitations in a prospeetive eohort study that included 12550 subjeets who did not have diabetes at the time of enrolment. The study was designed to examine the relationship between the use of antihypertensive medications and the risk of subsequent development of type 2 diabetes. The measured outcome was the development of type 2 diabetes mellitus, determined by fasting serum glueose coneentration, assessed at three and six years of follow-up. The results showed that subjeets with hypertension who were taking thiazide diuretics, angiotensin-converting-enzyme (ACE) inhibitors, or caleium-ehannel antagonists were found not to be at an increased risk for diabetes compared with those who were not using antihypertensive therapy. In contrast, hypertensive patients who were on beta-blockers had a 28 percent higher risk of subsequent diabetes compared to the eontrol group. Despite the potentially diabetogenie effeet of beta-bloekers, they have been proven to have signifieant long-term proteetive effects against cardiovascular events. Thus, physicians must assess the risk of developing of diabetes against the benefits of beta-bloekers in reducing eardiovaseular morbidity in hypertensive patients.
(Gress TW, Nieto FJ, Shahar E, Wofford MR, and Brancati FL. NEJM. (2000). 342: 905-912)

Genome of Drosophila Melanogaster has been Sequenced

(reviewed by Martin C. Chang, Ph.D., 0T3)

The genome of the fruit fly, *Drosophila melanogaster*, is now the second and largest genome of an organism to be sequenced. It eontains approximately 180 million base pairs (Mbp), of which most of the 120-Mbp euchromatie, gene-rich portion has now been determined. This is a landmark aehievement that marks the end of a century of gene hunting and heralds a new era of exploration and analysis. The *Drosophila* genome was completed as a eombined academic and industry effort, involving Berkeley, European, and Canadian projeets as well as the Celera Genomics Group. Requiring monumental technical effort, it was aeeomplished remarkably swiftly, with 75% of the sequence having been determined over the past year. The strategy used was whole-genome shotgun sequeneing, supported by extensive elone-based sequenee and a high-quality bacterial artifieial-ehromosome physieal map. Some gaps remain; however, the sequence is sufficiently aeeurate and eontiguous to be used for preliminary gene annotation and interpretation. The genome encodes ~13,600 genes, somewhat fewer than the smaller *Caenorhabditis elegans* genome, but with comparable functional diversity. The *Drosophila* genome has significant implications to research in human medicine, due to the conservation of biological proeesses from flies to mammals. Important mammalian genes that have homologs found in *Drosophila* can now be investigated using a whole arsenal of genetic techniques. Due to the fact that the *Drosophila* genome is compact and the number of genetie tools available, this sequence may be the Rosetta stone for deeiphering the human genome.
(Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG et al. Science. (2000). 287: 2185; Kornberg TB, Krasnow MA. Science. (2000). 287: 2218)

In the Media

Edward C.W. Leung, B.Sc. (0T2)

Death of a Child at Sick Kids Deemed Homicide

On February 24, 2000, the coroner's jury deemed the 1998 death of Lisa Shore a homicide. This finding of homicide implies that the six year old's death was a result of someone else's action.

Lisa was admitted to the Hospital of Sick Children on the evening of October 21, 1998 for reflex sympathetic dystrophy. She was put on morphine pump to control her leg pain. She was also on four other medications at the time, including Gabapentin, an anti-convulsant. She died the next morning from a complex drug interaction leading to cardiac and respiratory arrest.

The coroner's inquest discovered that the nurses responsible for Lisa, Ruth Doerksen and Anagaile Soriano, did not read or follow the doctors' orders. As a result, Lisa's vital signs were not monitored.

The Shore family lawyer, Frank Gomberg, also accused the Hospital of Sick Children of covering up the facts. His accusations included lost evidence, withheld notes, and erased tape-recorded notes.

The coroner's jury provided 35 recommendations to the hospital for improving the quality of care. The ruling also prompted the police to conduct new investigations into the possibility of launching criminal probes.

The Globe and Mail, February 25, 2000, A1, A16
The Toronto Star, March 7, 2000, B1, B7
The Toronto Star, February 27, 2000, A15
The Toronto Star, February 26, 2000, A3
The Toronto Star, February 25, 2000, A1, A3, B1, B2
The Toronto Sun, March 7, 2000, 10
The Toronto Sun, February 26, 2000, 7
The Toronto Sun, February 25, 2000, 4
The Toronto Sun, February 25, 2000, 5

Primary-Care Reform and Money Talks

During negotiations between the OMA and the Ontario government, there was intense speculation about whether the government would be able to include primary-care reform in the talks.

Federal Health Minister Allan Rock expressed his belief that "primary health care reform is fundamental to maintaining our health-care system as sustainable into the 21st century". In addition, he expressed support for Ontario government's effort to implement changes to primary care. Meanwhile, Ontario NDP Leader Howard Hampton argued that all family physicians in Ontario should be paid salaries and that the fee-for-service payment method should be banned.

The Coalition of Family Physicians claimed that doctors should settle for no less than an annual 10 per cent hike in fees to offset the increasing business costs of the past 10 years. The coalition accused the government of selling primary care reform as a solution to the emergency room crisis, which the coalition considered to be "a problem of not enough beds and not enough places to send chronic-care patients from our hospitals and insufficient community services."

Ontario Premier Mike Harris expressed that the newly negotiated schedules would be "reflective [of] or less than inflation." While the government is expected to implement "rostering" as soon as possible, it is suspected that the OMA will

demand rostered patients to face some sort of financial penalty to deter them from getting medical care from another doctor outside the primary-care network. According to the *Toronto Star*, the OMA wants "shared accountability" among patients, physicians, and the government.

However, the OMA's views are not shared by all its members. Family physicians in the organization are split over whether the fee-for-schedule system or the salary-based system is better. Some specialists in the OMA would prefer the fee-for-schedule system maintained with the billing threshold abolished. Meanwhile, residents and medical students would like to see financial penalties for new doctors practising in well-serviced areas abolished in these talks. It remains to be seen how the OMA can represent the disparate demands of its constituencies.

The OMA is also facing outside challenges. In a recent Globe and Mail publication, the newspaper discovered that the top billing family physician in Toronto billed $552,695.48 from OHIP. In fact, the top 10 billing amounts by family physicians in London, Ottawa, and Toronto all exceed $300,000. Duncan Sinclair, chairman of the Health Services Restructuring Commission, argued that such high billings would not occur if proper primary care reform was implemented.

While it is unclear what shape the final physician billing contract will take, the Ontario government seems determined not to repeat the 1997 negotiations where an extra $500 million was devoted to increasing physicians' salaries.

The Globe and Mail, January 15, 2000, A8
The Globe and Mail, February 22, 2000, A11
The Globe and Mail, March 28, 2000, A1, A5
The Toronto Star, January 15, 2000, A10
The Toronto Star, January 18, 2000, A6
The Toronto Star, January 19, 2000, A4
The Toronto Star, March 25, 2000, K1, K4
The Toronto Sun, January 14, 2000, 13

Alberta Health Care Protection Act (Bill 11) Faces Stiff Opposition

Alberta Premier Ralph Klein intends to pass the Health Care Protection Act (Bill 11) into legislation this spring. The bill will allow Alberta's 17 government-appointed regional health authorities (RHAs) to sign contracts with private clinics to perform minor surgical services, which are covered under medicare. The premier claimed that one of the benefits of the new legislation would be the shortening of line-ups for hip-replacement surgery.

However, it is up to the Alberta College of Physicians and Surgeons to decide what procedures health authorities can contract out. And since the announcement of Bill 11, the college has already indicated that hip replacement surgeries are probably too complicated for private clinics to handle as they involve considerable postoperative monitoring and invasive diagnostic procedures which require a full-service, public hospital.

The public is also weary of Klein's new health plan. While ophthalmological procedures performed in semi-private eye clinics are currently paid for by Alberta, cataract patients still pay for "extras", such as access to off-site diagnostic labs or special lenses at semi-private clinics. The discrepancy in service has already created some sentiments that a two-tier system already exists.

Moreover, the private system has not been shown to be more cost-effective or time-efficient. In Calgary where virtually all cataract surgeries are contracted out to private clinics, the wait is about 16 to 24 weeks. In comparison, in Edmonton where 80% of cataract surgeries are performed at public facilities, patients only wait five to seven weeks.

Canadian Union of Public Employees (CUPE) is also concerned that the Alberta's bill will give American health companies a legal means to erode Canada's medicare system under NAFTA.

Opponents of Bill 11 also pointed out that the total number of doctors and nurses are limited. Since doctors at private clinics are allowed to make more money by selling enhanced medical services under Bill 11, there is a concern that fewer doctors will be left in the public system.

While there are speculations that the government of Canada may withhold transfer payment to Alberta if Bill 11 were passed, Prime Minster Jean Chretien said, "He (Klein) can pass any bill he wants." Chretien explained that withholding of transfer payment under the Canada Health Act would occur if the implementation of Bill 11 led to the violation of the five principles of medicare.

Klein continued to defend Bill 11 by pointing out that similar legislations are in place in provinces such as Ontario, Saskatchewan, and Manitoba. In spite of the many setbacks, the premier seemed determined to pass the new legislation.

The Globe and Mail, March 4, 2000, A4, A11, A18
The Toronto Star, April 1, 2000, K1, K3
The Toronto Star, March 25, 2000, A10
The Toronto Sun, March 24, 2000, 2
The Toronto Sun, March 20, 2000, 7
The Toronto Sun, March 14, 2000, 24

Provinces Feud with Federal Government over Health Care Transfer Payments

Provinces demanding more health care funding from the federal government is likely not news to Canadians. Ever since the federal government announced the infusion of an extra

$2.5 billion into the provinces for health care, tension has been escalating between the federal government and the provinces.

The situation was worsened by the fact that the Ontario government decided to run an advertisement claiming that Ottawa currently pays only 11 cents of every health care dollar. Ottawa in return ran an opposing advertisement claiming that it spends 34 cents of every health care dollar in Ontario. The discrepancy lies in the fact that in 1977, Ottawa agreed to cut federal taxes by roughly 13 per cent so that the provinces can raise their taxes by a similar amount for health care. Ottawa still considers the revenues to be part of its health care contribution while the provinces do not.

A meeting gathering the provincial and federal health ministers was conducted on March 30-31, 2000. The provinces wanted Ottawa to restore the $4.2-billion in health care funding that it removed in 1994. Meanwhile, Federal Health Minister Allan Rock wants to ensure that any increase in federal transfer payment is attached to a commitment by the provinces to enact health care reforms. Rock wants to see the creation of a national home care program, primary care reform, and national standards on the delivery of health services. The provinces, on the other hand, are more interested in obtaining more health care money before committing to any specific plans of change.

Meanwhile, the federal government and the provinces are accusing each other of putting tax cut as a priority over increasing health care funding. Ottawa is also weary that any increase to health care funding will only lead to a reduction in the provincial commitment to health care and further provincial tax cuts. However, some fear that if Ottawa refuses to increase health care funding, the provinces will use it as an excuse to expand private health care or to delist existing services from medicare coverage. In this highly charged political climate, it remains to be seen whether any actual health care funding increases will come from either the provincial or federal governments in the near future.

The Globe and Mail, April 1, 2000, A8
The Globe and Mail, March 31, 2000, A6
The Globe and Mail, March 30, 2000, A4
The Toronto Star, April 1, 2000, A1, A10
The Toronto Star, March 31, 2000, A1, A24
The Toronto Star, March 30, 2000, A1, A6
The Toronto Star, March 29, 2000, A1, A7
The Toronto Sun, March 31, 2000, 10
The Toronto Sun, March 29, 2000, 36

St. Michael's is Canada's leading Catholic Academic Health Science Center and is fully affiliated with the University of Toronto. Located in downtown Toronto, St. Michael's provides a range of primary, secondary and tertiary services at the local, regional and provincial level. As a result of hospital restructuring, St. Michael's is one of three university teaching academies at the University of Toronto. Presently the hospital is located on two sites (Bond Street and Wellesley Street) with over 500 beds, and will consolidate to one site this year through an $80 million building and renovation program.

The hospital is organized in a programmatic fashion with emphasis on diabetes comprehensive care, heart and vascular disease, inner city health, minimal access therapeutics, neuro trauma and the musculoskeletal system. A strategic plan has recently been completed with a major emphasis on research and education. The new vision states the goal that St. Michael's Hospital will be internationally recognized as the finest academic healthcare provider in Canada.

CHIEF, PEDIATRICS

Pediatrics at St. Michael's Hospital is a newly created Department within the Inner City Health Program. Through hospital restructuring, St. Michael's was directed to establish a 20-bed Level II nursery that is currently being built in the new wing, along with a new maternity centre for 2,500 deliveries. The Departments of Obstetrics and Pediatrics are linked through the services of obstetricians, midwives, and family physicians through a wide range of primary and secondary services.

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Patricia Petryshen, RN, PhD
Vice President, Patient Care Programs & Chief Nursing Officer
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St. Michael's Hospital
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Toronto, Ontario M5B 1W8
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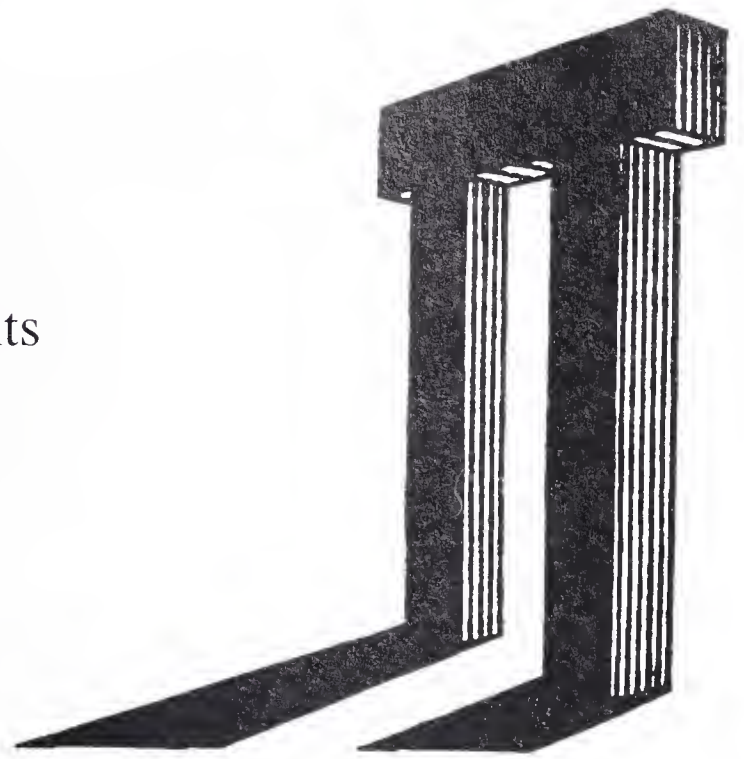

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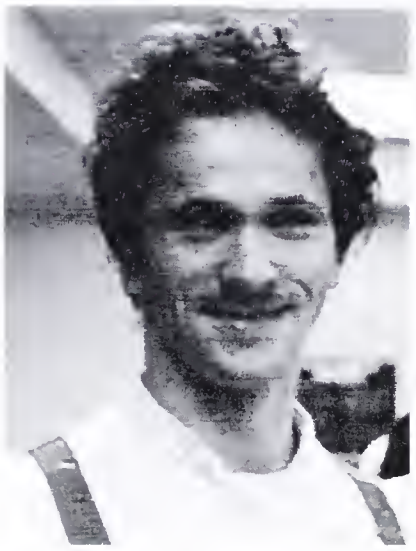
Prolific Scientist Profiles

Josef Penninger: From Heart Disease to Arthritis-What's Next?

Susan Quesnel, M.Sc. (0T2)



Dr. Josef Penninger is an Assistant Professor at the University of Toronto in the Departments of Immunology and Medical Biophysics. His research has made significant contributions to our understanding of cell fate in thymocytes, signalling in T cell selection and activation, and autoimmune heart disease. He was also recently recognized for his research in arthritis. His discoveries began when a researcher at Amgen in California cloned the osteoprotegerin ligand (OPGL) and suspected that it regulated bone metabolism by stimulating osteoclasts to eat bone away. Penninger's group subsequently created a mouse deficient for OPGL and observed that this mouse lacked osteoclasts completely. OPGL causes osteoclastogenesis by binding to its receptor (RANK) on chondrocytes, osteoclast precursors. Dr. Penninger was intrigued by the fact that OPGL also had some effects on the immune system, such as T cell function. When it was shown that OPGL is up regulated in certain T cells, he decided to pursue the study further.

Penninger: So the question we asked was why would the T eell, whieh fights infection, make the moleeule that eats the bone away? We established a new eulture system and all we need to do now is take a T eell, drop it on bone marrow eells and all of the cells become bone-eating osteoclasts. And further we could show that when the T eell is activated, it makes this molecule and it leads to bone loss. This was quite niee beeause nobody understood why, for example, people infeeted with Hepatitis, HIV, chronic autoimmunity, like diabetes, lupus, people with asthma, why do the develop bone loss? So every time you turn on the T cell, it's making this moleeule that eats the bone away. It was a moleeular explanation for many diseases, also for kids and adults with leukemia. Many of them are breaking their bones.

And then we moved further. Of eourse, a major disease is arthritis. In many eases of arthritis, it's the T eell that's initiating the disease. So we thought if this moleeule eats the bone away, maybe it has something to do with arthritis. We have this natural inhibitor in our body, whieh is ealled OPG (Osteoprotegerin-a deeoy reeeptor for the ligand). How the whole bone metabolism works is like a ear: OPG ligand (OPGL) is like the aeeelerator, you step on it and you lose bone, and OPG is the brake, you step on OPG and you brake bone loss. The balanee between them explains the whole thing. We ean explain post-menopausal osteoporosis in women now, because it turns out that estrogen regulates the brake. In menopause, estrogen goes down, the brake goes down, and you just step on the aeeelerator. After menopause, OPG goes down and OPG ligand works more. It's a very simple straightforward explanation. It also explains why women espeeially get osteoporosis rather than men beeause of the whole hormonal regulation of the system.

...So we can give rats arthritis, very severe disease. They are aetually eripped after fourteen days. After we gave these rats OPG on the days we see the first swelling, like a human patient that eomes to the doetor and says "my joints hurt", the amazing thing is that we ean eompletely prevent bone loss and eartilage loss, but not the inflammation. So this was really exeiting beeause if this is real, we ean wipe out eripping in arthritis. Of eourse, this was one model. But other people have repeated our experiments in other models and they all turn out to be exaetly the same.

N&V: Are you working on the clinical trials?

Penninger: Amgen is, yes. Also based on this data, they will use it for leukemia patients and things like that.

N&V: Will it be used to prevent osteoporosis as well as treating arthritis?

Penninger: Yes. This will be the biggest thing: to wipe out osteoporosis in twenty years. They're working on small molecule inhibitors, which would mimic the function (of OPG), the idea being that in twenty years, there might be just a pill that anyone can take, once a week, and prevent osteoporosis completely. This should work. This is the real thing. But this is only for the short term because in order to prevent it, we are treating healthy women. We have to be very sure that in twenty years none of them come down with breast cancer and things like this. So it will take a while before this gets on the shelf for women with osteoporosis.

I think in the short term, they will move very fast into rheumatoid arthritis, a severely crippling disease, and especially for cancer metastasis into the bone. Many women have breast cancer that has gone to the bone and eats the bone away. For them it works beautifully. Of course, you can't heal the cancer, but the problem for them is that they can't pick up the kids anymore because the bones are breaking. For these people, we should be able to completely shut down bone loss. I think it will probably really provide them with a better life. I think this is probably the first target population for this drug: people with breast cancer, prostate cancer, melanomas, and multiple myelomas. It will also be used for children with leukemia since most of those who survive have growth retardation because of bone loss.

And of course, rheumatoid arthritis will be a major target, considering that the U.S. is spending fifty billion dollars a year for arthritis. And if this really works, the system can save billions and billions of dollars...I get so many calls from people with arthritis hoping to get into clinical trials. I get calls from Europe and Australia; they would travel from there. It's because of OPG. Essentially, it means that there is really nothing out there. They want OPG to get on the market faster. This is the most amazing thing: to do something which has a real impact on people. If we could pull it off it will be such a beautiful thing.

Originally from Austria, where Dr. Penninger obtained his M.D. and Ph.D., he came to Toronto in 1990 as a post-doctoral fellow in Dr. Tak Mak's lab. He started his own lab at the age of 29, which he has successively run for the past six years. Today, his lab, at the Ontario Cancer Institute and affiliated with Amgen, consists of four graduate students, five post-doctoral fellows and three technicians. Like most scientists, a career in research was something that he never planned:

Penninger: When I was growing up, I didn't want to become a researcher. I wanted to become a painter or a mathematician. Theoretical physics and mathematics were my stuff. One afternoon, the sun was shining and everything was nice. I decided to become a medical doctor. I was sitting at the park and I thought I would become an M.D., which I did. I realized it's not the right thing for me. I don't have the personality to do that and I felt it was a little boring. So I did my Ph.D. in immunology. It's different in Austria, you can do it during your M.D. And I studied arts and Spanish at the same time. I really liked science. I never really thought there's actually a career doing science. Where I came from, all of the science is done by medical doctors at medical schools. So I actually didn't know until a long time later that you can actually have a job as a scientist. I came here to do post-doctoral studies with Tak Mak. In 1994, the Amgen Research Institute got started. I applied and got lucky. It was more an accumulation of chance events than a planned career. I never planned to do what I do now.

N&V: By now, you have had many offers from other institutions. Why do you choose to stay here?

Penninger: The first allegiance is to do good science. That's the most important thing. So most of the offers I got were to become a big Prof, some big shot somewhere. This is not what I want to be at this stage of my life. I don't want to be a big shot administrator. I want to do science. There are very few places in the world where you can do science like here, in this institute. The reason being that we get a reasonable amount of money, so we can do things that I like to do. It was really amazing because in the beginning, I had all of these great ideas when I started in the lab. I submitted all of these grant proposals to the Medical Research Council and the National Cancer Institute. Essentially all of my grants were rejected because, you know, you just don't do these things. I should stick to the stuff that I did and I shouldn't just dream up new things. I should just focus on one thing. I have no business to have these grand ideas. So all my grants were rejected. So without this place (Amgen), I would have dropped out of science three or four years ago. The good thing was that we really had enough money to just start projects even with these guys telling me that I'm

a megalomaniac and it's useless. It doesn't matter if I believe in it. So that was really nice. Also the culture of science here is great: all the downtown hospitals and the University. The people are very helpful. You can just call up somebody at the University and they'll give you more or less everything. There are very few people in town where you have to worry about them wanting to scoop you.

N&V: Do you think the Amgen Institute specifically provides the set up that you wouldn't be able to get elsewhere in Toronto?

Penninger: I think so, but it's not just our institute. It's a real small place in terms of numbers of investigators. We couldn't do much without being integrated into the Ontario Cancer Institute and collaborate with people at Mount Sinai and Sick Kids.

A *Nature* article published in January 2000, reveals another one of Penninger's breakthroughs. It describes the negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b.

Penninger: There's a big paradigm in immunology: To turn on a T cell you need two signals. One signal is the T cell receptor. The second signal is called the co-stimulatory signal. Only if you have signal one and two can the T cell do its trick and proliferate, fight virus infections, etc. It's very clear that you need these two signals. What's interesting is that Cbl-b is the gatekeeper of the second signal. It basically tells the T cell receptor, which is the first signal, to use the second signal to turn on the T cell response. When you mutate Cbl-b, the T cell receptor by itself can do it. You do not need a second signal anymore, which is quite amazing. And we could show it genetically. So you really do not need the second signal. And it's amazing because the net effect is every (knockout) mouse we have comes down with autoimmunity, inflammation all over the body, which makes sense. The idea being that we do not normally develop autoimmune disease because when our T cells go to our brain, the brain cells do not provide the second signal, only certain cells can do it, like in the spleen, in the lymph nodes, therefore we are kind of protected. Otherwise we would come down with diabetes every second day. The idea is that we don't get it because you need the second signal. So if you mutate the Cbl, you just get autoimmunity all over the place.

N&V: Was the involvement of Cbl in the immune system known before this?

Penninger: No, there were only three papers on the function of this molecule. And two of the papers said that it actually enhances the responsiveness and one said that it shuts it down. So it was completely unclear what this thing would do. And people missed it for a long time because most of the molecules were cloned out downstream of the receptors because they were tyrosine phosphorylated, so you could pull them out. This one is not tyrosine phosphorylated, so nobody knew that it was there in the first place. It's also interesting because for tumour immunology of most tumours that occur in humans, you can induce T cells to fight against them, but most of them actually actively tolerate the T cells because the tumours don't give them the second signal. The T cell just sees them, but so what. So in manipulating Cbl, you can turn the whole thing around and make the cells hot. All they need is to see the tumour.

N&V: Would this involve a gene therapy approach?

Penninger: Yes. Amgen is looking into this now. My involvement is now finished. One student works on this project and we just submitted a paper to science. We know now what Cbl is really doing at the molecular level. We have very strong genetic evidence that tells us what this thing is doing and why it's doing what it's doing. This should be quite interesting.

N&V: Is it difficult to have your own involvement end after all you've put into it?

Penninger: No. We tried to figure out the mechanism, but in terms of finding a drug or trying gene therapy - that's not my business. It's exploring new mechanisms, pathways, etc.

N&V: It seems beneficial to have it all organized by the same institute, from the basic science to the clinical trials.

Penninger: Yes. This is very important. I never really realized it, but it's really nice. To find something and actually have somebody behind you who has an enormous amount of money and can pull off things like that. It costs them three hundred million dollars to develop a drug in biotechnology. To develop OPG for osteoporosis costs them three hundred million dollars just to push it through the process. That's very expensive. So I can dream up things that could be very useful, but there are very few companies in the world that can actually pull these things off, who have the resources so that if something does not work, you can really work around it. Of course it's worth money in the end, it will be pushed very fast. They will try everything.

Especially for OPG, which is potentially a five billion-dollar drug.

Dr. Penninger has also made advances in studying chronic heart disease. Work done by his group has established how chlamydia infections can lead to heart disease by expressing antigens that mimic heart specific muscle protein. This leads to the production of autoantibodies to heart muscle epitopes and inflammatory heart disease.

Penninger: Very often you find inflammation in the heart and atherosclerotic plaques. They think a lot of chronic heart disease might be based on autoimmunity. We started with viruses, which infect and destroy the heart muscle. Then you get the immune response. In some animals, you actually find no virus anymore, but the animals develop severe chronic heart failure. So there was some idea that acute virus infection in the heart muscle would trigger autoimmunity. Then we mapped the peptide in the heart muscle that does it and looked to see if this peptide looked the same as these viruses. It turned out that it looked the same as chlamydia, which was quite nice because chlamydia had been implicated in hundreds of papers that it could lead to heart disease. But all the papers would just find the bug in the blood vessel wall of people with heart disease. How do you know what's the cause and effect. What if these guys are just innocent bystanders? In atherosclerotic plaques, you not only find chlamydia, but many other bacteria. How do you know which one is doing what and when, etc? So we had this mimicking peptide and shot it into the mouse and they all came down with inflammatory heart disease. At least it's a direct cause-and-effect relationship. We call it Marla 7a because the guy who started it, his girlfriend's name was Marla. We are actually studying more bacteria and we think that chlamydia is just the beginning of the story. There are many bugs out there, which can probably do the same thing. Of course the real question is for how many humans will this be relevant?

His interest in immune-mediated heart disease began with the coxsackie B3 virus (CVB3), which can be found very often in patients with chronic heart failure (30-50%). If this virus is expressed in mice, they all come down with severe acute heart disease and inflammatory heart disease. He thought the chronic failure would be caused by autoimmunity. Although it turned out that the mechanism for CVB3-induced heart disease does not involve autoimmunity, Penninger has identified the essential host factor (p56lck) that is essential for the replication and pathogenicity of CVB3.

Penninger: So in this case, it's more a frontal attack of the virus against the heart. Instead of the chlamydia protein mimicking our own heart muscle protein, in this case, the virus goes into the heart, destroys half of the heart, and of course our T cells have never seen this heart muscle protein before, and now they just start attacking. So it seems infection might cause very many cases of heart disease or chronic heart failure. And the reason also is that in 50% of patients with chronic heart failure, they don't have any risk factors. They're perfectly healthy and they drop dead of a heart attack. In many of them, you find these viruses. It's not the only one, but it's probably the most frequent one.

...People are calling me and saying " I have a cold, am I going to get a heart attack?" Of course, that's the implication. When you find the virus in 30-50% of these patients, it means this virus must strike very frequently. Probably every time it infects us, it causes a mild myocarditis, which you would never detect.

N&V: So what's next on your agenda?

Penninger: We are now actively looking for new genes that regulate heart development in flies. Now we have the whole fly genome and within half a year, we'll have the whole human genome. If there are mechanisms that are critical for the development of cancer, they tend to be conserved in evolution, so the same would be true for flies as for humans. So we are searching for these genes in flies, because it's very easy to set up genetic screens. And if we find them, we could go straight into the humans knowing essentially every gene. We already hit on two nice ones, which probably regulate blood pressure and things like this and they look very interesting. We also found out the real function of the bone-eating molecule, OPG ligand. So I think osteoporosis is more or less an evolutionary accident. The real thing is completely different. We submitted the paper two weeks ago. This was really surprising. It turns out that it's essential to form a lactating mammary gland during pregnancy. Without OPG ligand, there's no milk protein production, there's no formation of lactate in the mammary gland, so all the pups are dying. What's really neat is that kids get calcium by drinking from the mothers. So calcium is absolutely critical for brain and heart development. So the same molecule that sucks calcium up from the bones is the same molecule which forms the organ to bring calcium to the next generation to keep the species alive. This is also regulating calcification of arteries in old people, most likely. We have the whole thing and it's real, we have the whole pathway figured out, receptors and everything. So it turns out that the early pregnancy hormone turns on OPG ligand directly in the breast epithelium and then it signals via a receptor called

RANK, for the cells to survive and proliferate to make more cells to make the milk protein. If we don't have OPG ligand, we are completely blocked in differentiation. We know it's a direct effect because we can put back OPG ligand in one breast of a knockout mouse, but not the other one. And this one is completely normal when the mouse gets pregnant and it's a knockout. So there's a direct straight effect on the epithelium. Of course many women have to take calcium during pregnancy and lactation because OPG ligand sucks it out. It makes complete sense. I like it. I think that's the real function of it.

N&V: How do you pick what areas to target in your research?

Penninger: I think to pick a project is the most critical thing. If somebody comes into my lab, I tell them to sit down for two months and really think about what they would like to do and then discuss it with everybody. Kind of a democratic process, but I decide at the end. It's more about the process, "what you personally would really like to do and how would this fit in with what other people in the lab are doing." Also, would this make a real difference? Will it be important to people?

For arthritis, it was more that we had found the T cells regulating osteoclastogenesis, that they can lead to systematic bone loss when you turn on T cells and then we wanted to see if the same thing happens during local bone loss, which is critical for crippling arthritis. This was just the last part of the puzzle: to take arthritis as a model system to prove our point. We didn't study arthritis, we were led to it. For heart disease, there is a very strong personal interest. My mother had severe strokes and heart disease. She's been in the hospital for six years. Since I have the chance to do a little, I really wanted to do something. My lab might seem very large to some people, but its very small compared to some big shots in the US, who have labs of 40-50 people who study heart disease. So we have to come up with some real little clever ideas to be competitive against these guys; make some twists and turns and not just follow the main stream, but study things which we find interesting and try novel approaches.

During the interview, Dr. Penninger alluded to many other important studies underway in his lab, many of which are soon to be published. As mentioned above, he has recently elucidated another role for OPG ligand in lactation, or as he puts it "the real function." He has also identified a new tumour suppressor involved in colon cancer. The list could go on. At this rate, it is possible that the most exciting discoveries made by Josef Penninger are yet to come.

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Prevalence of Violence in the Canadian Health Care Workplace

Elana S. Lavine, B.Sc. (OT2)

The year 2000 was ushered into the city of Toronto by both festivity and tragedy. Late on the evening of December 31st 1999, Henry Masuka was shot and killed by the emergency task force of the Toronto police. This father of four had arrived at the emergency department of St. Michael's Hospital, accompanied by his asthmatic son. Reportedly hysterical and adamant that his son be treated immediately, Masuka pulled a pellet pistol modelled after a semi-automatic handgun and put it to the neck of an attending emergency physician. Shortly after downtown Toronto began its viewing of a millenium fireworks display, the emergency task force decided that Masuka could not be calmed, and opened fire.[1]

Although the details of this incident will likely be debated for some time it is, at the least, another reminder of the frightening and often unpredictable role that violence has come to play in health care settings. While occupational risks in medicine are known to range from the transmission of infection to the sequelae of stress and strain, it may be important for physicians, nurses, and medical trainees to increase their level of awareness on violence as an occupational hazard.[2] The contemporary usage of the term 'violence' encompasses not only the classic picture of an attack fueled by fury or rage, but also any aggressive behaviours which cause harm or discomfort to intentional targets or innocent bystanders. Violence may include physical assault, harassment, or threats of harm.[2] The focus of this article will be on physical assault and the statistics available which help to frame the current picture of its prevalence in Canadian health care.

Incidence

The level of physical violence directed against Canadian health care workers is increasing. While the rate of Workers' Compensation claims lodged in British Columbia more than doubled between 1982 and 1991, the number of claims lodged by health care workers in that province quadrupled. As of 1998, nurses in British Columbia faced an equivalent risk of workplace violence as police officers, with an incidence more than four times the incidence of any other profession.[3] From 1987 to 1989 in Ontario, Workers' Compensation allowed over 100 claims among nurses for injuries due to violence. The annual rates for such claims were 13.9 per 1000 for male nurses, and 1.4 per 1000 for female nurses; these are both significantly higher (54 and 5.9 times higher, respectively) than that of the general Ontario work force. Such compensation data likely underestimate the true extent of this issue, because no statistics are available for denied claims or claims with no lost time, and many assaults are unreported.[4] While the British Columbia Medical Association is the only provincial body of physicians to have a formal Committee on Violence, a visit to the website of the Ontario Medical Association yields a list of current, high-profile quick-reference topics, which includes "Violence against physicians." [5]

Risk Factors

The risk assumed by any health care provider may vary according to the time of day, the site of work, the particular field of medical practice, and the types of patients with whom he or she interacts.

Nursing Staff

The apparent majority of literature on violence against health care providers is published in nursing journals. Violence is seemingly well documented as a recognized risk of the nursing profession, and this documentation includes strategies for risk assessment, management of violent patients, and supportive approaches for nurses who have been victims of violence while on the job.[6, 7]

Indeed, a published survey of 808 registered nurses working in Ontario reported that 59% of nurses had been physically assaulted at least once by patients while working, with 5% of

those physical assaults resulting in severe injuries.[8] Another retrospective 1994 study of a large, inner city hospital in Winnipeg found that of the 242 reported abuse-related injuries, 194 (80.2%) occurred among the nursing personnel, with the highest rate of injury found among psychiatric ward nurses.[9]

Physicians

The murders of several physicians who provided therapeutic abortions, and the enormous media frenzy that followed, led to a series of events including the formation of a Canadian National Task Force on Doctor-Related Shootings. In a memo to the Canadian Medical Association, a detective in the Ontario Provincial Police subsequently detailed ten security steps for physicians, which included examining backyards for irregularities and reporting strange persons or vehicles to the local police.[10] An editorial published by William Orovan, president of the OMA at the time, included a sentiment of concern for physicians driving alternate routes to work, drawing curtains while at home, and living in general fearfulness.[11] This entire cluster of events and the corresponding press releases and articles, were fairly specific to those physicians involved in legal abortion services.

The behaviour of physicians may be under increased scrutiny and attack from both patients and institutions, which represent the public interest. This creates demands that are difficult for physicians to meet. Physicians who become defensive or overly self-protective from these overwhelming pressures may further offend their patients, increasing public anxiety, outrage, and even the incidence of violent retaliations.[12] In the Winnipeg study mentioned above, only 1.2% of abuse-related injuries were reported by physicians.[9] However, the author suggests that this likely represents a lack of reporting by the medical profession, and at this particular hospital, physicians were poorly covered by the occupational health program, as they were not considered "employees" of the hospital and not required to observe corporate policies and procedures applicable to all other staff.

Hospital Risk Factors

Academic medical centres are often focal points of workplace violence within their universities, with the incidence of violence peaking at night. Hospitals may be sites of increased risk for violent attacks due to such factors as numerous unsecured entrances and exits, extended hours of operation, the nature of the surrounding community, the gender and legal obligations of the workforce, their size, as well as continuous staff exposure to potentially violent situations.[13] The sheer volume of visitors to hospitals, the presence of overnight visitors, dimmed corridors, empty patient rooms, and the challenge of effective security have also been cited as contributing factors.[7]

A recent study examined 993 violent incidents, over seven years, at a highly ranked American teaching and research university. It found that a medical environment (in this case, the associated teaching hospital) increased the likelihood of a work-related violent incident by over seven times.[14] Fifty-three percent of all work-related violent incidents observed in the study occurred in the teaching hospital with the largest number of these incidents occurring between 6 p.m. and 6 a.m. Another American study retrospectively analysed one year of reported incidents at another university teaching hospital, and found that one-third of all episodes occurred between 11 p.m. and 7 a.m.[15]

Patient Risk Factors

Medical factors that might predispose a patient towards violence include head injuries, seizures, psychiatric disorders, diabetes, intoxication, drug overdose, drug or alcohol withdrawal, and post-traumatic stress syndrome.[13] Staff working in close contact with such patients need to be appropriately prepared for the early identification of a "pre-violent" state, as evidenced by verbal or physical cues, and trained in intervention and management of such situations in order to avoid escalation to violence.[13]

Medical Specialty Risk Factors

Psychiatric wards, emergency departments, admitting areas, and long-term care facilities have been identified as the medical environments associated with the highest risk of violence against nurses.[14] Violence in emergency wards has been well documented, and is still believed to be grossly under-reported. Factors that may exacerbate the risk of violence include the stresses of illness and its acuteness, long waiting times, the availability of drugs, the availability of potential hostages, a 24-hour open door, and the presence of psychiatric patients.[16] Emotions such as uncertainty, anxiety, and fear may engender stress and anger, which often lead to violence.[7] In addition, in the current environment of health care cutbacks and resource limitations, patients often present to emergency departments with misled expectations of accelerated access to investigations and therapies; when those expectations cannot be filled, anger and frustration might be directed toward those persons most likely to be nearby, namely physicians and nurses.[17] A recent study of 106 emergency department employees at an inner-city tertiary care centre in Vancouver reported that 57% of staff had experienced physical assault while working in the emergency department in 1996, with the highest proportion of assault victims being nurses and protection services personnel.[18]

Violence in psychiatric hospitals was recognized as an issue of concern earlier than in other settings, and is the subject of much research. In our aging society, the documented incidence of physical assault against health care providers in geriatric hospitals and nursing homes will likely be the subject of increased attention. Abuse of staff at long-term care facilities is frequent, and may be linked to residents' disabilities such as brain injuries, dementia, and chronic progressive diseases.[19]

Violence Against Postgraduate Medical Trainees

The issue of violence directed against the medical resident has been explored in the recent literature. A 1996 study of sexual, psychological, and physical abuse in the residency training programs at McMaster University found that physical assault during residency training was reported by 19.6 percent of 184 respondents. Of these reports, 14.7% involved male patients and family members as perpetrators, and 9.8% involved female patients and family members. Most of the remaining incidents of assault were committed by supervising male physicians; these incidents were reported by 5.4% of female residents and by none of the male residents. This difference may reflect a true discrepancy between male and female residents as the victims of physical assault from their superiors, or an underreporting of such events

from male residents, perhaps due to denial or rationalization.[20] Data published in 1991 found that 40 percent of Canadian psychiatric residents reported physical assault at least once in the workplace, resulting in requests for improved security by 37 percent of the respondents.[21] A survey of 475 surgical residents in the U.S. found that 179 (37.7%) had been physically attacked, with most incidents occurring in the emergency department, wards, or parking lots. Female residents were more likely to call hospital security for intervention in potentially violent situations, and junior residents were attacked more frequently than senior ones.[22]

Conclusions

Health care, and the challenges it faces, continue to mirror the changes in Canadian society. Although medical professionals have chosen careers with great responsibility for the health and welfare of society, they have likely also unwittingly volunteered for an increased risk of physical assault compared with the majority of the Canadian population. Such violence is most commonly recognized as stemming from interactions with patients and their families; however, violence between superiors and their trainees may require further assessment.

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Nanomedicine and Nanotechnology: Speculative Frontiers

Martin C. Chang, Ph.D. (0T3)

"Nevertheless electric telegraphy must have greatly diminished the number of letters, for new improvements now permitted the sender to correspond directly with the addressee...The telegraph network now covered the entire surface of the earth's continents and the depths of the seas; America was not more than a second away from Europe..."

Jules Verne, 1863.[1]

The prophetic voice of Jules Verne often strikes today's readers as visionary. Yet, Verne was a writer of fiction, imagining what Paris would be like in the 1960s, based on the emerging industries of the 1860s. Some argue that in ways, his writings were self-fulfilling. Yet the manuscript of "Paris in the Twentieth Century" was only recently published, having languished in storage for over a century. The more Romantic souls would ask, "What if...?" What if this manuscript were treated as a model for future technology, and set upon by the scientists and engineers of the day? What if Verne himself was an ambitious and well-funded scientist? Would we have had fax machines by 1920? E-mail by 1950? Such speculations are, of course, not the domain of "serious" scientists. Or aren't they? Today's spiritual successors to Jules Verne are both idealists with a vision and accomplished scientists. They are the devotees of nanotechnology.

Nanotechnology: Definition and Approaches

"Nanotechnology" is a term that is both widely misunderstood and misused. It is often used incorrectly to describe the miniaturization of known devices. It is often confused with the closely allied field of quantum computation, which requires devices small enough to exploit quantum states to handle information. True nanotechnology, or "molecular nanotechnology," concerns the building of structures on an atom-by-atom basis, to exact and complex specifications.[2] Put simply, nanotechnology is the theory of controlling the atomic structure of matter: manufacturing using atoms themselves as building blocks. The pedigree of nanotechnology is illustrious. Richard Feynman casually discussed the lower limits of scale for manufacturing machinery in 1960 at an after-dinner talk.[3] The true pioneer of nanotechnology is generally acknowledged to be Eric Drexler, an interdisciplinary scientist whose doctoral degree from the Media Lab at MIT was self-designed. Drexler is the author of nanotechnology's seminal text, *Nanosystems*, and founder of the Foresight Institute, a California-based think-tank which actively promotes nanotechnology.

Drexler's approach, which he calls "theoretical applied science," is distinctive and an anathema to many scientists and engineers. Drexler's interest, and that of fellow nanotechnologists, is neither in determining how to accomplish an advance nor in revealing a novel aspect of nature. Rather, what is important in nanotechnology is the use of known physical laws to describe the properties of a hypothetical nanotechnological device. This helps determine the lower limits of performance for entire classes of nanodevices that may exist (e.g. bearings, gears, motors).[2] These devices are typically geometric arrangements of small atoms (C, N, O, H, S, P, Si, Cl, F are the most important) that are drawn using a molecular modelling software package. Once drawn, the molecular orbital properties for the given arrangement are calculated, and factors such as bond stiffness and oscillations taken into account. These nanodevices are closest in structure to diamond: a natural geometric lattice of carbon atoms covalently bonded with remarkable strength. As a result, nanodevices are described by Drexler as "diamandoid."

Small diamandoid devices are modelled by computer and then used to form components for larger hypothetical devices. For example, molecular models for bearings, gears, and motors are designed and then stringently evaluated to meet set lower limits of performance. Given the soundness of the nanocompo-

nents' designs, it would theoretically be possible to build a nanocar. Moreover, the emphasis is placed not on producing a single optimal design, but to show that a whole class of different designs are workable, and that once manufactured, they will perform to a predictable set of specifications. What is scientifically unusual about this approach is that it does not add to an understanding of physical law, neither do any of the tools required for building these devices exist. Physically, these devices cannot yet be tested. Chemically, carbon lattices such as C_{60} and other fullerenes have been created, but the devices described by Drexler have a more complex degree of organization. Their manufacture requires a high level of control over atomic trajectories.

Building Sky Castles?

Critics of nanotechnology, which include most funding agencies, understandably protest that such theorizing does not actually help to advance current technologies.[4] Biochemists are already designing enzyme-mediated reactions to produce custom molecules. Protein engineering allows the manufacture of highly organized and predictable molecular structures. Yet, the manufacture of nanodevices requires reactions that are optimally performed under high vacuum while shielded scrupulously from cosmic rays. Drexler himself concedes that in a world where science and engineering pursue ideal priorities, nanotechnology's theoretical approaches can only occupy a fraction of the effort devoted to pure theoretical science (which enhances understanding of physical laws), experimental science, and engineering.

In response to these criticisms, nanotechnologists take two distinct (but not mutually exclusive) positions. The first is the view undoubtedly shared by Jules Verne, that an understanding of potential technology helps prepare humanity for its advent. Many beneficial applications of nanotechnology can be envisaged. For example, the ability to custom-arrange atoms will eventually allow kilogram quantities of food to be produced from sludge composed of carbon, oxygen, nitrogen, hydrogen, sulfur, and phosphorus – a solution to world hunger, together with its associated social dilemmas. Charles Platt of Wired magazine has published an online "Nanotechnology Museum" in the style of Jules Verne, offering a vision of the future with nanotechnology: from nanocomputers to nanorobots capable of creating space habitations. Nanodevices could have numerous medical applications, as described below. Conversely, nanodevices that could create food and shelter can also destroy. To have such technology emerge without some consideration of its consequences could be dangerous indeed. One of the activities of Drexler's Foresight Institute is to examine the social adjustments that will be needed to manage nanotechnology, and to determine the institutions that must be established in order to effect these adjustments. By opening up discussions of nanotechnology, keeping as much of the work in the public domain as possible, a democratic environment for ideas is established – ideas that will be necessary in a world with nanotechnology.

The second position is more practical: that a multidisciplinary understanding of nanotechnological principles will lead to a gradual increase of goal-oriented activities in the requisite fields of chemistry, physics, biochemistry/protein engineering, macromolecular engineering and micromanipulation techniques. Since the publication of Nanosystems in 1992, the nanotechnology community has campaigned tirelessly for multidisciplinary interest. The Foresight Institute hosts yearly meetings, bringing together enthusiastic researchers from all the requisite fields. The last meeting, held at Santa Clara, California in October 1999, brought together researchers in molecular electronics, biomolecular engineering, scanning probe microscopy, computation, chemistry, robotics, and materials science. The keynote speaker was none other than the University of Toronto's beloved and ubiquitous John Polanyi, 1986 Nobel Laureate for Chemistry, a pioneer in the study of chemical reaction dynamics. Nanotechnologists therefore reply to their critics that by encouraging accomplished experimentalists, theoreticians, and engineers to pursue their own interests while thinking about nanotechnology, the means to produce nanomachines will come with time. And when it does, there will be machines already designed, and their implications already examined.

Nanomedicine: Designing Healing Machines

While many of the physical and computer scientists who pioneered nanotechnology dreamed of building quantum computers and terraforming asteroids, Robert A. Freitas, Jr. was dreaming of medical applications for the new technology. Holding degrees in physics, psychology, and law, Freitas is the author of the newly published *Nanomedicine: Basic Capabilities*, the first in what is to be a three-volume textbook. *Nanomedicine* is also the first, and as yet only, thorough examination of potential nanodevices in the human body and the resulting social and clinical implications. Freitas, like other nanotechnologists, thinks big. While many clinicians are looking for the next effective treatment, Freitas looks for nothing less than the Fountain of Youth: "Once nanomedicine becomes available, we'll each [live long enough] to pursue our longest-term and most interesting goals [in life]." Science fiction? For the moment, nanomedicine is but a distant dream. Yet, physical laws allow for the existence of devices that will perform clear medical tasks. The description of these devices is the main pursuit of nanomedicine.

While more practical medical technologies focus on protein design and recombinant gene products, nanomedical machines will, like typical nanomachines, be diamandoid, composed mainly of carbon, but using other light elements where special properties are needed. A number of factors affecting the potential success of introducing such devices are examined by Freitas. These include the interaction of the devices with human body fluids and immune factors, power sources, the need for remote control, removing the devices from the bloodstream, and targeting to tissues. The eventual goal will be a highly controlled population of nanomachines residing within the body, "keeping house" and constantly repairing damage. For now, nanomedical advocates focus on designing simple machines with simple clinical functions.

The nanomedical device that has been most thoroughly described is Freitas' "respirocyte," an artificial mechanical red blood cell. The respirocyte is a spherical nanorobot made of

18 billion atoms, mostly carbon atoms arranged in a lattice structure.[2] This crystal lattice forms a diamandoid chamber that is pumped full of CO_2 and O_2 molecules. In keeping with the principles of nanotechnological design, this complex model is based on the modelled performance of simpler components. On the surface of each respirocyte is a cohort of almost 30,000 molecular sorting rotors, originally described by Drexler.[2] Each rotor is a spinning wheel with receptors for CO_2 and O_2, and either brings in extracellular CO_2 in exchange for O_2 (as a red cell would at the periphery), or vice versa (as in the alveolar capillaries). Whether the respirocyte gains CO_2 or O_2 would be governed by onboard computers* linked both to the sorting rotors and to chemoreceptors for CO_2 and O_2. The respirocyte therefore can have the oxygen-exchange curve of hemoglobin in a red cell, except the strong diamandoid chamber would have a carrying capacity exceeding 200 times that of a real red cell. Respirocytes would replace the need for transfusions and would have the curious effect of allowing a person supplemented with 500 mL of these cells to stay at the bottom of a swimming pool for 2 hours![6]

Other currently known nanomedical designs have diverse functions. An anti-cancer nanobot has receptors (based on protein structure) that recognize cancer cells, and a chamber containing an anti-cancer drug that is injected directly into the cell. Nanoscale surgical operations from within, using computer-controlled nanobots have been proposed, as has the use of nano-imaging robots to reveal in vivo anatomy. One problem with the specific design of a medical nanobot to solve a present clinical problem is the possibility that concurrent research in other areas will render the nanobot obsolete before it exists. For example, if cellular and genetic processes are developed to shut down cancer cells, the advent of an anti-cancer nanobot will provide an ax for a scalpel's job. This highlights the importance of interdisciplinary approaches when considering nanotechnology and nanomedicine.

Sensible and Speculative Paths

When will nanodevices become a reality? Some experts estimate that it might take only 20 years for the manufacturing processes to become workable. Others believe it will take much longer. Even critics of nanotechnology begrudgingly admit that it may come into being around the dawn of the 22nd century. This timeframe is still too rarified for most scientists, engineers, and clinicians. Most therefore view nanotechnology more as a curiosity. Yet, there seems to be a storm of excitement brewing amongst a growing community of researchers in many disciplines. The atmosphere is heady, with talk of "a new revolution" and "global abundance." Let us pursue our "sensible" paths: a clinical education free of such wild speculation. Let us pursue our own diverse interests. But let us also be aware of the speculation around us, and of the promise it holds.

Further Reading

Interested in learning more about nanotechnology? The first site to visit is the Foresight Institute homepage at: www.foresight.org. This site offers a wealth of articles and essays on nanotechnology and suggests other readings.

For those with a background in the physical sciences or engineering, Drexler's *Nanosystems* offers a complete overview of the field. Freitas' *Nanomedicine* is best viewed as an update-companion to *Nanosystems.*

For those more interested in a non-technical account, Drexler's *Engines of Creation* (Doubleday, 1986) was the first popular work on the subject, whereas Ed Regis' *Nano* (Little, Brown and Co., 1995 - available at U of T Engineering library) is more impartial.

* A nanocomputer controlling the respirocyte need not be as sophisticated as the integrated-circuits that control our cars. Nanotechnology aims only at workable designs, and not optimal ones. Such a computer would be based not on electron flow, but on mechanical switches and gates, reminiscent of Babbage's difference engine. Freitas' respirocyte control chip is a mechanical system with a speed of about 1 kHz (10,000 times slower than the first IBM PC). Such a speed can be accomplished with a relatively simple nanocomputer simply because of the small scale.

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Malnutrition in the New Millenium

Abha A. Gupta, M.Sc. (OTO) and Monica Gupta, B.Sc.

As Sister Agnes and I walked through the lanes of Sunder Nagiri *basthi* (slum), we came upon a boy sitting on the narrow path, inches away from the open sewer, picking at whatever was next to him – a half-eaten cob of corn, a *beedi* butt (a local unfiltered cigarette). It was difficult to say where his mother was – perhaps on the roof cooking, washing clothes, or breast-feeding her other child who was only one year younger.

With the help of the neighbours, we found the boy's mother, Saira, and asked her what she was feeding her children. She was exclusively breastfeeding her 18 month old, with no additional supplementation. Her answer was remarkably similar to that which we had heard in the first ten homes visited earlier that day.

Saira's youngest two children were significantly underweight, falling into third degree malnutrition, which is defined as only 60% of the weight of a normal child of the same age. We plotted the two boys on the growth charts made for each child in the *basthi*, and tried to explain using the curve that her children should be at least 2 to 3 kg heavier than they were. We counselled her to supplement her baby's diet with solid foods such as vegetables, dhal (lentils), and chapattis, as well as monitor more closely what the older one was eating.

Malnutrition remains a significant problem among Indian children. Fifty percent of the boys and 64% of the girls between the ages of 1 to 5 years living in the Sunder Nagiri area were underweight. The major causes of malnutrition identified in the area include the use of diluted formula with breast milk in the first six months of life, delayed complementary feeding to 8-10 month olds, high incidence of gastrointestinal disease, undernourished mothers, large families, and discrimination against female children.

When offering nutritive counselling, we were occasionally met with some resistance, or at least indifference to our suggestions, as mothers argued that the regimens they used to feed their older children seemed to be 'fine.' Their children eventually grew, even if not to the body mass index we are accustomed to North America. Saira herself was an anemic 4 ft 11 inches. Mothers seemed satisfied with the quality of their children's health, having only their immediate neighbours' children for comparison.

Saira had already experienced the death of a child at one year of age from severe diarrhea. The child mortality rate (under 5) of Sunder Nagiri is approximately one in ten, similar to the Indian national average of 99 per 1000 live births in 1996.[1] It is not uncommon in Sunder Nagiri for a family to lose a young child, providing parents an increased incentive to have more children just in case of such a catastrophe.

Maternal education is known to be significantly associated with a child's nutritional status.[2] In Sunder Nagiri, more than 80% of the mothers had absolutely no formal education. However, in addition to maternal education, total family income, paternal education, family hygiene, and religious customs likely play contributive roles in a child's nutritional status.

Lack of education seems to allow the perpetuation of customs and tradition that may not always be in the best interest of the child. The mother-in-law of one woman forbade breastfeeding of her newborn child for the first few days of life, until the

colostrum changed into whole milk. It was believed that the colostrum was dirty, 'like pus,' and only after appropriate cleansing of the breasts and development of whole milk should the baby be allowed to feed.

Sister Agnes was one of many employees at the St. Stephen's Hospital (Delhi, India) community health centre which offered many health and education programs to the inhabitants of Sunder Nagiri. During my stay, they began a Malnutrition clinic, specifically for severely malnourished children, where detailed dietary histories were taken and appropriate recommendations were provided. Some mothers were invited daily for directly observed feeding to ensure their children were receiving adequate caloric intake. The poverty in Sunder Nagiri was staggering, but the lack of knowledge and motivation among the populace to change the status quo was even more alarming.

As described in the United Nations Declaration of the Rights of the Child in 1959, a child has a right to free education, to learn to be a useful member of society, and the right to enjoy nutrition, housing, and medical care. The intricate relationship between health, nutrition, and education necessitates a holistic approach to the intervention. The hope is that one can begin to help instill motivation to attain higher standards of living among the people of such communities, and break the cycle of poverty. As we enter a new millennium, it should be a priority for the international health community to help fulfill these most basic of human needs for our future generations.

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The Ever Changing Face of Modern Medicine: Adults with Congenital Cardiac Defects as a New and Emerging Population

Manish Sood, B.Sc. (0T2)

"In science generally to solve one set of problems may be to create or discover a whole new set, and of no science... is this more true than of medicine."

– George P. Elliott[1]

Adults with congenital heart defects (CHD) are one of the most successful rehabilitation stories ever witnessed by medicine.[2] Medical, surgical and diagnostic advances in cardiology and cardiac surgery have allowed for children with CHD – historically a fatal condition – to live into adulthood. This has created the rapid expansion of a new patient population that requires specialized novel therapies and management. Congenital cardiac lesions are exceedingly complex, yet the importance of an understanding of their relevant anatomical, physiological, and clinical manifestations cannot be understated. As this new population matures into adulthood, numerous medical and psychological complications unfold. The medical community is beginning to recognize this population and progress is being made towards optimizing their management and care.

Cardiac surgery is a contemporary discipline, which began tentatively with the suture of the heart in 1896 by Ludwig Rehn of Frankfurt.[3] Exploration into congenital heart anomalies was soon to follow, Maude Elizabeth Abbott (1869-1940), a Canadian anatomist and graduate of Bishop's University, collected and catalogued over one-thousand cases of hearts with congenital defects and was the first to understand their physiologic effects.[4] Surgical correction of such deficits, however, was a much more daunting, and risky, task. Robert E. Gross, a surgical resident at Boston Hospital, undertook the epochal procedure of ligating a patent ductus arteriosus in 1938 and introduced a new era of curative cardiac surgery. He was soon followed by Helen Taussig and Alfred Blalock, who in 1944 sutured the left subclavian artery to the pulmonary artery (the Blalock-Taussig procedure) to aid children with cyanotic heart disease.[5] Subsequent advances such as the heart-lung machine, cardiac catheterization and the development of open-heart surgery greatly extended the boundaries of treatment postnatally. Fetal echocardiography, which determines the anatomic development of the heart and vessels as well as allows detection of subtle abnormalities in blood flow, turned intrauterine diagnosis of congenital heart defects into a reality.

The rapid advances in diagnostic methods and in the medical and surgical care of infants born with congenital defects allows 85% of such patients to live into adulthood.[6] It is estimated that in Canada alone, there are between 60,000 and 75,000 adults with CHD.[7] This population is extremely diverse, including those who have never undergone cardiac surgery, those who have had cardiac surgery and require ongoing management, and those in palliation, awaiting heart transplantation.

It is of the utmost importance that the cardiac lesion be recognized and characterized in order to plan an appropriate treatment and management regime. The assessing physician should look for key criteria of the patient's lesion such as the presence of cyanosis, pulmonary hypertension, the origin of the lesion (left or right-sided) and the dominant ventricle. Diagnostic tools include ECG, chest X-ray, echocardiography (with or without Doppler), and cardiac catheterization. Tables 1 and 2 provide a classification of common cyanotic and acyanotic cardiac lesions, respectively, with the associated anatomic/functional changes.

Shunting of systemic venous blood to the arterial circulation results in reduced arterial oxygen saturation, which is

Table 1
Classification of Common Cyanotic Congenital Cardiac Lesions with Anatomic Abnormality and Subsequent Physiologic Changes[8]

Type of Lesion	Anatomic Abnormality	Physicologic Changes	Signs and Symptoms
Tetralogy of Fallot	Large ventricular septal defect, overriding aorta, obstruction of the right ventricular outflow tract, right ventricular hypertrophy	Right to left shunting which varies with systemic vascular resistance	Hypoxic spells with tachypnea, hyperpnea, loss of consciousness, seizures and CVA; clubbing, cyanosis, palpable right ventricular lift, single S2, aortic ejection click, systolic ejection murmur, on CXR – classic boot-shaped heart
Ebstein's anomaly	Tricuspid valves septal and posterior leaflets are displaced into the right ventricle and the anterior leaflet is malformed; small right ventricle; 80% have concurrent ASD	Severity depends on degree of displacement and functionality of valve leaflets; if severe elevated right atrial pressure and right to left interatrial shunting	Highly variable – usually fatal in infancy; adult presentation is due to a supraventricular arrhythmia (paroxysmal atrial tachycardia) with cardiac failure, syncope, brain abscess and sudden death; severity of cyanosis varies, widely split S1, S2 with S3, S4, systolic murmur due to tricuspid regurgitation
Transposition of the great arteries	* D-transposition: aorta arises from the anterior right ventricle and the pulmonary artery arises from the left ventricle	complete separation of the pulmonary and systemic circulation; need a patent ductus arteriosus and/or foramen ovale for communication	Severe cyanosis and tachypnea; non-specific findings – loud S2, right ventricular hypertrophy
Eisenmenger's syndrome	Atrial septal defect, ventricular septal defect and/or patent ductus arteriosus	Pulmonary vascular obstructive disease leading to obliteration of the pulmonary vascular bed and a right to left shunt	Cyanosis, exertional dyspnea, palpitations, hemoptysis, CVA; clubbing, elevated JVP, right parasternal heave, loud P2 , S4

** D-transposition – refers to a complete transposition where the aorta arises from the right ventricle and the pulmonary artery arises from the left ventricle. There is no overriding of the great vessels and essentially no mixing of blood between the two circuits unless there remains a patent ductus arteriosus and/or foramen ovale.*

the basis of cyanosis in CHD. Children and infants with cyanotic lesions require surgical correction to allow for survival into adulthood. For example, adulthood survival rates (>32 years of age) for patients with Tetralogy of Fallot increased from 4 to 86% following complete surgical correction in childhood.[9, 10]

In general, acyanotic lesions can be highly insidious but early recognition remains fundamentally important as this type of CHD may pose complications in late adulthood. Such is the case in the patient with a bicuspid aortic valve, as the complications such as sclerosis of the valve will typically become symptomatic by the fifth decade of life.[12] Atrial septal defects (ASD) are the most common adult congenital heart defect, accounting for approximately one-third of all cases. The more serious ventricular septal defect (VSD) commonly presents in childhood and infancy. With the exception of severe VSDs, patients with acyanotic lesions commonly survive well into adulthood.

The medical complications posed by CHD in adults are numerous with several emerging common themes. Pulmonary hypertension is almost universal, with the pulmonary vascular compliance being the principal determinant of clinical manifestations and possible treatment regimens. Therefore the quantification of pulmonary hypertension and more importantly, the comparison between pulmonary and systemic flow and resistance warrant careful evaluation. Long-term increases in pulmonary blood flow, pulmonary arterial pressure, and pulmonary venous pressure as well as polycythemia, systemic hypoxemia, and acidosis have been implicated as the etiology of pulmonary vascular obstructive disease (PVOD).[13] There is no proven treatment for PVOD, a severe disorder where permanent pulmonary vascular necrosis occurs; however, heart-lung transplantation has shown promise.

Erythrocytosis, an increase in the number of circulating red blood cells from erythropoietin elevation in the face of chronic hypoxemia, is another common complication. It is

Table 2
Classification of Common Acyanotic Congenital Cardiac Lesions with Anatomic Abnormality and Subsequent Physiologic Changes[10]

Type of Lesion	Anatomic Abnormality	Physicologic Changes	Signs and Symptoms
Atrial septal defect	Ostuim primum, ostuim secundum, sinus venosus	Interatrial shunting (degree is determined by size of defect and ventricular compliance; shunting is usually left to right)	Dyspnea on exertion, fixed splitting of S2, 'soft' early systolic ejection murmur
Ventricular septal defect	Inlet septum, muscular septum, perimembranous septum, infundibular septum	Intraventricular shunting (degree is determined by the size of defect and relative systemic and pulmonary resistence; shunting may be left to right or vice-versa)	Highly variable – small defects may be asymptomatic while large defects may have left ventricular failure or right ventricular failure associated with pulmonary hypertension
Patent ductus arteriosus	Failure of ductus arteriosus closure	Left to right shunting from the aorta to pulmonary artery	Asymptomatic if small;if large fatigue and dyspnea ; bounding peripheral pulses, widened pulse pressure and hyperdynamic left ventricular impulse; continuous 'machinery' murmur
Aortic stenosis	Discrete subvalvular, valvular, supravalvular	Dilatation of the aortic root, left ventricular hypertrophy and dilatation	Angina pectoris, syncope, pre-syncope and heart failure; delayed carotid upstroke, inaudible A2, S4, systolic crescendo-decrescendo murmur
Pulmonary stenosis	Subinfundibular, infundibular, valvular, supravalvular	Right ventricular systolic pressure increase with a systolic pressure gradient between the right ventricle and pulmonary artery	Asymptomatic if small while if large, fatigue dyspnea and eventual right heart failure with peripheral edema and ascites; palpable right ventricular impulse with thrill, wide S2 splitting, crescendo-decrescendo systolic murmur which increases with inspiration, ejection click

routinely seen in cyanotic patients and may lead to symptoms of recurrent hyperviscosity, including impaired coronary perfusion, headache, fatigue, dizziness, and thrombocytopenia.[14] Treatment is therapeutic phlebotomy that may lead to a host of complications, such as iron deficiency and a further decrease in oxygen delivery to tissues.[15]

Renal function, along with urate metabolism, is often abnormal in CHD patients. Low renal excretion of uric acid due to enhanced reabsorption is the cause of the hyperuricemia. Invariably, the chronic elevation of urate and its by-products lead to an increased risk of gouty arthritis and arthralgias. Renal histopathology shows enlarged hypercellular glomeruli, basement membrane thickening, focal interstitial fibrosis, tubular atrophy, and hyalinization of both the afferent and efferent arterioles.[16]

A major concern and complication in CHD patients is the dramatic risk of developing infective endocarditis (IE). In this disease, there is an infection of the endocardium along a natural or prosthetic heart valve, septal defect, or intracardiac patch. IE carries a high rate of mortality, hovering between 10 and 20%.[17] CHD patients are susceptible to IE due to their cardiac abnormalities, which lead to turbulent flow, or the presence of prosthetic valves. Many patients require lifelong antimicrobial prophylaxis along with lifestyle modifications, such as meticulous hygiene practices with dental and skin care.

The focus of previous research was directed towards physiological alterations, surgical or medical treatment, and the potential complications of CHD. However, from the patient's viewpoint, the impact of this chronic condition upon his or her life is equally concerning. Many specific issues need to be addressed such as pregnancy, exercise potential, and the existence of co-morbid conditions such as anxiety or depression. Brandhagen *et al.* (1991) found that congenital heart patients are more likely to have difficulty facing anxiety-provoking situations, to feel more

susceptible to illness, and are less likely to obtain a university degree.[18] This population requires not only medical and surgical therapies, but also psychosocial intervention. Further investigation is warranted as there is a lack of research addressing this issue.

Presently the care for most adult CHD patients is delivered at multidisciplinary, specialized tertiary medical facilities, such as the Congenital Heart Disease Center at the Toronto Hospital. However, as this patient population expands, it will become crucial that adequate management be offered by regional care centers, individual specialists, and primary caregivers. In March 1998, the Canadian Cardiovascular Society (CCS) put forth recommendations on the diagnosis and treatment of adults with congenital heart defects, acknowledging the special needs of this patient population.[19] The aim of these recommendations – the first of their kind – is to inform and educate a broader spectrum of physicians about congenital heart disease in adults.

It is essential for physicians and the general health care community to acknowledge and adapt to the adult patient with a congenital heart defect. Although the advances made thus far are favorable, effort must now be directed towards the development of effective long-term care strategies that will address the needs of this new and emerging population.

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Law and Ethics in Medicine

Ask the Expert

Anna A. Kulidjian, M.Sc. (0T2)

This series is intended to serve as an introduction to legal and ethical issues in medicine. It features articles from experts in the field, as well as from medical students and trainees. The goal of this series is to clarify important legal and ethical issues before they become concerns at the bedside.

In this last issue for the year we are answering the questions we have received from you, our readers. We thank those of you who send these very interesting questions, and hope that next year many more of you will participate in this interactive section of the series. Please direct any comments, questions, or suggestions regarding this column to the Series Editor, Law and Ethics in Medicine at the following e-mail address: ediors.utmj@utoronto.ca

Scenario 1
TO TELL OR NOT TO TELL...

A physician in a hospital diagnoses an elderly man with cancer. A man is fully competent but does not speak English well. Citing cultural differences, his family demands that physician not tell the patient of his true diagnosis, as this diagnosis will cause fear and suffering in his last days of life. Is the physician "free" to decide either way, i.e. to tell or not to tell the patient?
– OT2

Bernard M. Dickens, Ph.D., LL.D.
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The first duty of the doctor is to protect the patient's health and integrity. The family may help the doctor to understand the type of person the patient is, but they are not disinterested, and may indeed have a conflict of interest. They say that the patient cannot cope with knowing his diagnosis, but it may also, or alternatively, be that they cannot cope with him knowing.

The danger of the doctor simply accepting the family's demand is both legal and ethical. The legal danger is that the patient's consent to accept treatment will not be adequately informed, which, if injury results, may expose the doctor to legal liability for negligence, based on breach of the duty of proper disclosure. The ethical danger is that, in supposing that the patient cannot cope with knowledge of his diagnosis, the family are being patronizing or paternalistic; they are reducing the elderly patient to a child-like status. Further, they may be denying him the power, in anticipation of death, to put his affairs in order, both materially and emotionally. That is, he may want to pay financial and emotional debts, capitalize his various assets, and say farewell to his family members, even at the cost of causing them to confront their own emotions about him. The doctor should not be party to the family members' paternalism.

The doctor may want to attempt to transcend the language barrier through an independent (non-family-member) translator, and to form an independent opinion of how well, or whether, the patient can cope with the knowledge of his diagnosis and the care the doctor proposes. It is valuable that the family has alerted the doctor to the possibility of the patient's inability to cope, but the doctor owes it to the patient to reach an independent decision. The family cannot demand nondisclosure.

If the doctor independently concludes that information of the diagnosis would be harmful or countertherapeutic for the patient, the doctor can accept the legal responsibility to invoke the "therapeutic privilege" of nondisclosure of information that patients should in principle be offered. The courts construe this privilege very narrowly, for fear that an exception to disclosure may destroy the duty of disclosure itself. Part of the doctor's clinical skill, and training, should be in giving a patient the bad news of an adverse diagnosis. The privilege cannot be invoked to permit the doctor to escape a difficult, emotionally draining task. The privilege may be invoked, however, if the doctor concludes, in competent clinical judgment, that presenting the diagnosis to the patient would compromise the patient's care, and/or

be a source of separate injury. The privilege cannot be invoked for fear that, with true information, the patient may make choices the doctor considers ill-advised.

Accordingly, the doctor is not free to withhold this patient's diagnosis from him, unless convinced in clinical judgment, based on reasonable grounds, that disclosure would compromise his necessary care, or be a source of positive psychiatric harm to him. This patient is mentally competent, and any basis of deliberately treating him paternalistically, that is of infantilizing him, will need the strongest justification. The family's citation of cultural traditions in the country from which he came, will in itself be insufficient. An initial approach may be for the doctor, through appropriate translation, to speak to the patient to gain a sense of what extent of information he wants of his diagnosis, prognosis and proposed treatment. The family may have to be educated into the Canadian medical practice of treating competent adults with respect for their autonomy.

Scenario 2
MUST DOCTORS BE GOOD SAMARITANS?

Can a physician be charged for not helping the victims of crash they witnessed, or can they be charged it they try to provide medical help, but do not succeed in saving the victim, or accidentally harm the victim?
– OT2

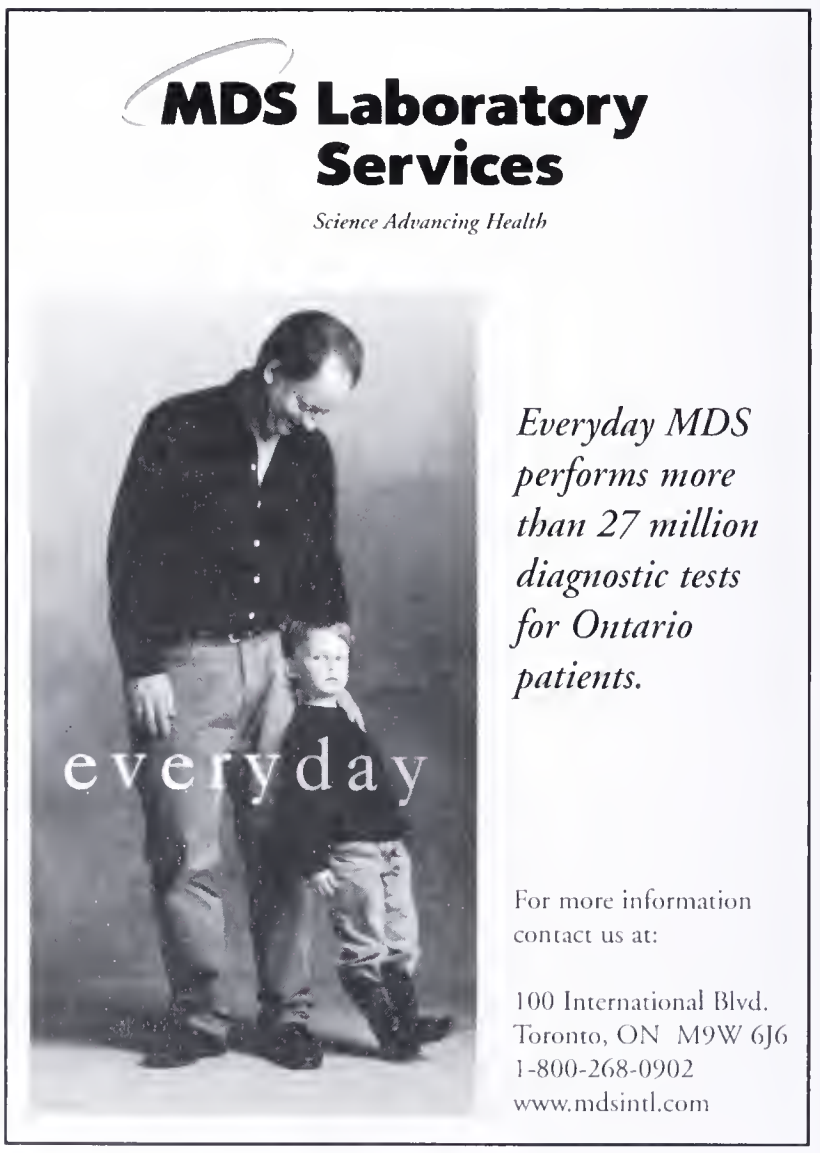
Laws differ on doctors' responsibilities to offer assistance to strangers, such as those doctors find injured in traffic accidents. The rule that there is no legal duty that doctors owe to assist people they have not accepted as their patients applies generally in North America and other countries that follow the (Anglo-Saxon) Common law tradition. The French-based Civil law tradition, however, imposes a duty of reasonable rescue. This applies in Quebec, and other jurisdictions may have enacted legislation that creates a comparable duty. Like everyone else, doctors have duties in emergency to assist persons under their care, such as their dependent family members, and victims of their own driving or professional errors.

A doctor who chooses to give assistance at the scene of an accident that occurs among strangers need not be over-concerned about victims' consent. The legal principle is that "peril invites rescue," so that reasonable efforts to give comfort, to preserve health and to improve prospects of recovery have victims' implied consent. Consent does not have to be medically informed; conscious victims need only consent to be aided by a person entitled to say "I am a doctor." Consent cannot be implied, of course, where victims refuse offered assistance, unless they clearly show dementia or other mental incapacity. Courts have held a doctor liable, for instance, for transfusing an unconscious Jehovah's Witness who carried a card refusing blood and blood products, even though his purpose was to reduce risk of death.

In order to encourage well-intended assistance, especially in the event of sudden or imminent peril when best judgment may be compromised, the law relaxes the standard of care normally expected of those who voluntarily take responsibility to help. They are not held liable for failing to meet the standard of care set by the law of negligence. They may be liable only for what is described as "gross negligence," which means negligence that is so glaring that no-one of reasonable sense and perception would act in that way. The standard is calibrated to the type of person giving assistance, so that a non-physician may not be considered grossly negligent for an action that it would be grossly negligent for a doctor to do, for instance concerning whether or how an injured person is moved.

More problematic is whether doctors can charge fees for rescue initiatives. In that interventions have victims' consent, billing their public or private health insurers may be legitimate, but once doctors propose to treat consenting victims as patients, they assume full professional responsibility, and are governed by the regular law of medical negligence. Further, victims willing to have their health insurers billed may not expect to have to make personal payments, and may deny that they intended to accept an offer to assist on a contractual basis, so that they assumed no reciprocal duty to make even reasonable payment. If a doctor belongs to a professional association that requires altruistic aid, billing may be excluded.

It is lawful for Canadian doctors outside Quebec, to decline to give assistance to endangered strangers, even when requested. The doctor's response to an emergency is therefore governed not by law, but by professional and personal ethics.



Evidence-Based Medicine

How to Read an Article on Risk

Evette Weil, B.A. (0T2)

This article, the sixth in the *UTMJ* series on evidence-based medicine (EBM), examines the initial steps in the implementation of EBM into everyday clinical practice. The goals of EBM are to encourage physicians to ask clinical questions, seek answers from the best available evidence in the literature, and integrate this evidence with their clinical expertise and their patients' values.

Introduction

As the emphasis in our health care system increasingly shifts toward the goal of preventative medicine, physicians are often faced with identifying patients who will likely develop a disease from those who will not. This interpretation of risk is based on studies that (seek) to find a statistically significant relationship between measured variables and particular outcomes. To continue the *UTMJ* series on evidence-based medicine (EBM), this article attempts to help medical students and physicians understand how to read an article on risk factors, focusing particularly on univariate and multivariate regression. These statistical principles will be illustrated with the following case example:

Case Study

C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women[1]

This case-control study of post-menopausal women sought to demonstrate that inflammatory markers, such as C-reactive protein (CRP), could be used to identify patients at high risk for cardiovascular events. In total 12 markers were evaluated. An event was defined as death from coronary heart disease, nonfatal myocardial infarction or stroke, or the need for revascularization. The study found that CRP was the strongest univariate predictor of risk of cardiovascular events. Additionally, analytical models that included markers of inflammation and lipid levels were better at predicting cardiovascular events than models of lipid levels alone. The authors concluded that CRP was a valuable predictor of cardiovascular risk in post-menopausal women.

Regression

In the case example, the investigators were interested in ascertaining whether serum measurements of inflammatory mediators could be used to predict future cardiovascular disease. They used the analytical tool of regression to determine the relationship between the markers and later events. In statistical language, the inflammatory markers represent the explanatory or independent variables, and the outcomes represent the target or dependent variables. The goal was to see whether increased inflammatory markers were found more often in patients with disease than in those without disease, and whether any difference could be attributed to more than simply chance.

The study evaluated the serum measures of 12 markers in 122 case subjects who developed cardiovascular disease and 244 control subjects who did not. The first analysis was a simple or univariate regression. A univariate regression is an analysis in which each independent variable, such as serum CRP levels, is evaluated separately from all of the others. As can be seen in Table 1, the relative risk of having a cardiovascular event increases as the serum levels of CRP increase. In fact, the relative risk of suffering an event is 4.4 times greater in patients in the highest group compared to patients in the lowest group. The small P value suggests that there is only a minimal chance that the association between CRP and cardiovascular disease can be ascribed to chance.

Table 1
Univariate Analysis of Increasing CRP Levels

CRP	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P Value
Median (mg/dl)	0.06	0.19	0.38	0.85	
Relative risk	1.0	2.1	2.1	4.4	<0.001

It is interesting to note that there is a higher incidence of cardiovascular events with increasing CRP levels; but is this information useful to physicians? The medical community has already identified multiple risk factors, including age, smoking, hypertension and lipid levels. Do CRP levels tell us anything new? This question can be answered in two ways.

A univariate analysis can compensate for some confounding variables by selecting appropriately matched control subjects. In this study, controls for each case subject were selected based on matching age and smoking status. Therefore, the study design eliminated these two variables as sources of variation. However, it is impossible to select subjects matched for every desired variable. Instead, when comparing two patient populations with different characteristics multivariate regression allows one to isolate those variables that provide additional information.

Multivariate regression simultaneously considers all variables to determine the relative predictive ability of each variable.[2] A variable is considered to be an independent predictor of risk if on multivariate analysis, relative risk is still considered significant. In our case example, an analysis which took into account all 12 of the markers and other risk factors (hypertension, diabetes, parental history of myocardial infarction) showed that elevated CRP levels remained an independent predictor with a relative risk of 1.5 (P=0.02). This means that elevated CRP levels provide the physician with information useful above and beyond that provided by other known risk factors. In contrast, other inflammatory markers that were significant predictors in the univariate regression, are no longer significant in the multivariate model (Table 2). The loss of predictive power occurs because the inflammatory mediators are strongly associated with each other: one inflammatory stimulus is likely to increase all the markers. Therefore, the serum levels of CRP are known, knowing the levels of other markers does not provide additional information.

Table 2
Comparison of Univariate and Multivariate Regression Results

Variable	Univariate RR	Univariate P value	Multivariate RR	Multivariate P value
CRP	4.4	<0.001	1.5	0.02
Serum amyloid A	3.0	0.002	1.1	0.4
sICAM-1	2.6	0.004	1.1	0.6
IL-6	2.2	0.02	0.8	0.2
Total cholesterol: HDL	3.4	<0.001	1.4	0.02

(RR, relative risk, sICAM-1, soluble intracellular adhesion molecule type 1; IL-6, interleukin-6; HDL, high density lipoprotein.)

Table 2 also shows that, in addition to elevated CRP levels, an increased ratio of total cholesterol to HDL cholesterol is an independent predictor of cardiovascular events. How is the clinician to interpret these results? Since elevated CRP and the cholesterol ratio give supplementary information, it can be inferred that a patient with both markers elevated is at greater risk than a patient with one marker elevated who is in turn at greater risk than a patient without elevations in either marker. This contention is supported by other analyses in the study. For example, tests of likelihood ratios demonstrated that predictive models incorporating both lipids and CRP levels were more telling than models based on lipids alone. So the clinician reading this article may decide to measure both the lipids and CRP in select patients to more effectively stratify patients at risk.

Conclusion

As part of the continued desire to prevent illness and improve target therapy, clinicians are often required to calculate the risk of disease for a particular patient. In keeping with the principles of evidence-based medicine, physicians must be able to interpret published articles on risk factors. An accurate interpretation is dependent on a thorough understanding of the analyses used to define predictive variables; namely, univariate and multivariate regression. With knowledge of these analyses, the physician can reliably choose the appropriate diagnostic tests and thereby improve their abilities to foretell future events.

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Back to Basics III

The Complete Blood Count and Peripheral Blood Film

Stephen C. Juvet, B.Sc. (0T2), Yvonne Chan, M.Sc. (0T2), and Ian Quirt, M.D., F.R.C.P.(C) (6T9)

This is the third instalment of a three-part series in which essential diagnostic tests of key importance in medicine are reviewed.

Introduction

In this third instalment of the Back to Basics series, we turn our attention to one of the most commonly ordered laboratory investigations in medicine: the complete blood count (CBC) and the peripheral blood film. The CBC is an important component in the diagnostic work-up of many types of illness including infections, inflammatory diseases, bleeding disorders, and cancer. Additional information can be obtained from microscopic examination of the peripheral blood film, particularly when the latter reveals cells specific to particular disease processes (e.g. sickle cell anemia), and a number of situations in which the CBC results alone are insufficient to establish a diagnosis. In most clinical settings, a technologist examines the peripheral film and a brief report accompanies the CBC results. Where they are instructive, some examples of peripheral films are included where they are instructive. Figure 1 is a normal peripheral blood film.

It is assumed that the reader has a basic knowledge of the hematopoietic system, the various cell lineages, and their functions. Normal adult CBC results are shown in Table 1; actual reference ranges vary somewhat between laboratories.

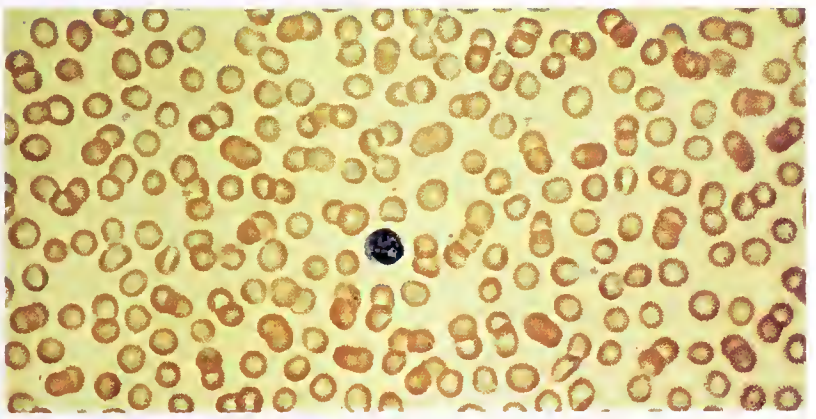

Figure 1. *A normal peripheral blood film. The red cells have a uniform, biconcave shape. Note the presence of a normal neutrophil, which serves as a guide: its nucleus should be approximately the same size as a red cell (in the range of 6-9 μm).*

Table 1
Normal Adult Complete Blood Counts

Measurement	Normal Males	Normal Females
Hemoglobin (Hb)	13.5-17.5 g/dL	11.5-15.5 g/dl
Erythrocytes (RBC)	4.5-6.0 x 10^{12}/L	3.8-5.2 x 10^{6}/L
Hematocrit	0.40-0.52%	0.37-0.47%
Mean Corpuscular Volume (MCV)	80-96 fL	
Mean Corpuscular Hb (MCH)	27-32 pg	
Mean Corpuscular Hb Concentration (MCHC)	31-36 g/dL	
Reticulocytes	25-85 x 10^{9}/L	
Leukocytes (WBC)	4-11 x 10^{9}/L	
Neutrophils	2.0-7.5 x 10^{9}/L	
Lymphocytes	1.5-4.0 x 10^{9}/L	
Monocytes	0.2-0.8 x 10^{9}/L	
Eosinophils	0.04-0.4 x 10^{9}/L	
Basophils	< 0.1 x 10^{9}/L	
Platelets	150-400 x 10^{9}/L	

Note that in modern laboratories, the hematocrit, MCH, and MCHC are all calculated on the basis of the Hb, erythrocyte count, and MCV. In general, therefore, these indices have limited use and are not discussed further in this article. We will begin with a discussion of red cells, proceed to white cells, and conclude with platelets.

Step 1. Consider Red Blood Cells.

The hemoglobin (Hb) concentration is a measure of the content of Hb, in g/dL, in the blood. Hb is found within erythrocytes and binds oxygen and carbon dioxide. It is a tetrameric protein

consisting of two α and two ß chains, with a heme prosthetic group. The Hb concentration is frequently considered first in examining CBC results. A low Hb concentration indicates **anemia**. Anemia becomes clinically significant when the delivery of oxygen to tissues and clearance of carbon dioxide become impaired. The signs and symptoms of anemia (Table 2) can result from the underlying cause or from the effects of the anemic state itself.

Table 2
Symptoms and Signs of Anemia

Symptoms	Signs
Fatigue	Pallor (especially conjunctival)
Malaise	Tachycardia
Dyspnea	Wide pulse pressure
Palpitations	Systolic flow murmur
Syncope	
Headache	
Angina (with underlying CAD)	

There are many causes of anemia; however, the various anemias can be classified into three broad groups on the basis of the **mean corpuscular volume (MCV).** The MCV, a standard component of the CBC, is a measure of the average volume of the patient's red cells.

Low Hemoglobin and Low MCV: Microcytic Anemia

Low MCV (< 80 fL) anemias are called **microcytic anemias** because the patient's red cells are of a smaller average size than normal. There are only two major causes of microcytic anemia, **iron-deficiency anemia** and **thalassemia**. They can be differentiated on the CBC by considering the **red cell distribution width (RDW).** In both normal and pathological states, there is some degree of variation in the sizes of red cells in the peripheral blood. The red cell volume distribution is a curve along which the different red cell sizes fall according to their frequency in the blood sample. The width of this curve, measured along the horizontal (cell size) axis, is the RDW.

- In iron-deficiency anemia, the RDW is increased. This reflects the fact that in most iron-deficiency states, the amount of iron available for erythropoiesis varies from time to time. Hence, red cells of many different sizes are found, although the mean red cell size is smaller than normal.
- In thalassemia, a genetic deficiency of one or more globin alleles is the cause of the anemia. In any given individual, the impairment in erythropoiesis is constant. As a result, the RDW is normal, indicating that red cell sizes are tightly distributed about the mean.

Iron-deficiency anemia is the most common form of anemia in developed nations. Most frequently, the cause is depleted iron pools as a result of chronic blood loss. However, acute blood loss can also produce iron-deficiency anemia if sufficient iron is lost in this manner and not replaced therapeutically. Some of the causes of iron-deficiency anemia are:

- Physiologic causes: menstruation, infancy, adolescence, pregnancy, lactation
- Chronic GI bleeding: hemorrhoids, diverticular disease, peptic ulcer, NSAID therapy, colon carcinoma, etc.
- Malabsorption
- Post-gastrectomy
- Dietary lack (rarely the only cause): infant on cow's milk, special diets, etc.

Chronic GI bleeding should be considered the most likely cause in an adult male or post-menopausal female. Patients with iron-deficiency anemia may have the symptoms and signs listed in table 2 as well as brittle hair, koilonychia (spoon-shaped nails), atrophic glossitis (smooth-surfaced tongue), angular stomatitis, and pica (an appetite for strange substances). In iron-deficiency anemia, the peripheral blood film shows microcytic, hypochromic red cells with some pencil forms and target cells (see Figure 2).

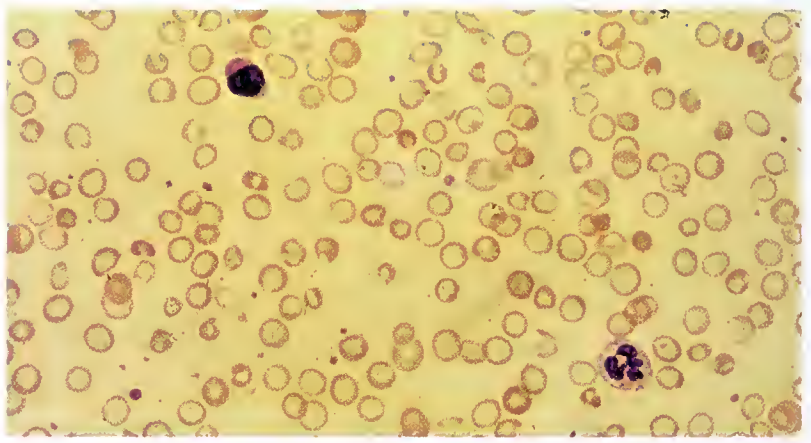

Figure 2. *Iron-deficiency anemia. Compared with the red cells in figure 1, these red cells are smaller (microcytic), less pigmented (hypochromic), and there is a greater variation in size.*

The thalassemias are a group of genetic disorders of the globin genes in which globin synthesis is impaired. They are common in certain ethnic groups (Italian, Greek, Chinese, Black). In the human genome, there are two ß-globin loci and four α-globin loci. The severity of thalassemia depends on the number of defective alleles:

- **ß-thalassemia** minor results from a defect in one of the two ß-globin alleles. The resulting impairment in Hb synthesis causes a microcytic anemia that is mild and usually not clinically significant.
- **ß-thalassemia** major results from a defect in both ß-globin alleles (the individual is homozygous for the defective allele). Clinical presentation occurs as fetal hemoglobin (HbF) is replaced by adult hemoglobin (HbA). Presenting symptoms include stunted growth, severe microcytic anemia, and gross hepatosplenomegaly resulting from extramedullary hematopoiesis. Regular blood transfusions are required to sustain life.
- **α-thalassemia** falls along a spectrum from silent (one defective allele), α-thalassemia trait (two defective alleles), HbH disease (three defective alleles), and Hb Bart's (all four alleles defective). HbH disease results in excess α chains, leading to Hb composed of four ß globin chains (HbH). HbH is unstable, and causes a microcytic anemia presenting in adults. The peripheral blood film shows red cell inclu-

sion bodies consisting of precipitated HbH. Similarly, Hb Bart's is composed of four γ globin chains, which is physiologically ineffective. Hb Bart's results in severe congestive heart failure and generalized edema of the fetus, leading to intrauterine death (hydrops fetalis).

It should be kept in mind that mixed genetic disorders of the globin genes can occur (e.g. an individual having one ß thalassemia allele and one sickle cell allele). Furthermore, in some cases it will be impossible to distinguish thalassemia and iron-deficiency anemia on the basis of the RDW. This can occur when the RDW is in the high normal range, and requires examination of the peripheral film to confirm the diagnosis. It is of course possible for thalassemia and iron-deficiency anemia to coexist.

A third and much less common cause of microcytic anemia is **sideroblastic anemia**. Sideroblastic anemia represents a group of hereditary and acquired disorders in which abnormal heme biosynthesis prompts elevated iron uptake by red cell precursors. This leads to the accumulation of iron in these precursors. The hereditary form is very rare; acquired forms may result from exposure to toxins and certain drugs, and in some individuals may herald the onset of leukemia. The diagnosis of sideroblastic anemia requires a bone marrow aspirate.

Low Hemoglobin and High MCV: Macrocytic Anemia

Anemia in the setting of a high MCV indicates **macrocytic anemia,** that is, the patient's red cells are larger than normal. Very large red cells (MCV > 110fL) indicate **megaloblastic anemia**, in which red cell maturation is prolonged owing to a lack of nucleic acid precursors. The cause of megaloblastic anemia is almost always a deficiency of the nutrients folate and/or cobalamin (vitamin B_{12}). In general, dietary deficiency of folate must have been present for 4 months, and/or dietary deficiency of cobalamin must have been present for 10-15 years, for megaloblastic anemia to develop. Table 3 summarizes the major causes of folate and cobalamin deficiency.

Table 3
Causes of Folate and Cobalamin (B_{12}) Deficiency

Causes of Folate Deficiency	Causes of Cobalamin Deficiency
Dietary (common)	Dietary (uncommon)
Alcoholism	Strict vegetarianism
Infancy	(10-15 years)
Poverty	Gastric
Intestinal	Mucosal atrophy of
Malabsorption	pernicious anemia
Drugs	Intestinal
Methotrexate	Malabsorption
Alcohol	Stagnant bowel
Anticonvulsants	Parasitic worm infection
Oral contraceptives	Post-ileal resection
Increased Needs	Genetic (rare)
Pregnancy	
Prematurity	
Hemolytic anemia	
Psoriasis, exfoliative dermatitis	
Hemodialysis	

Pernicious anemia is an autoimmune disease in which gastric parietal cells are destroyed, leading to reduced secretion of intrinsic factor, a protein that binds cobalamin and carries it to its site of absorption in the distal ileum. This leads to a chronic lack of cobalamin, and results in megaloblastic anemia.

A variety of drugs can produce megaloblastosis, including methotrexate (a folate antagonist), certain antineoplastic agents (6-mercaptopurine, 5-fluorouracil), and sulfonamides (folate analogues). Oral contraceptives and anticonvulsants (e.g. phenytoin) can also have this effect. The peripheral blood film in megaloblastic anemia shows oval macrocytes (Figure 3a) and neutrophil hypersegmentation (nuclei with more than five lobes, Figure 3b).

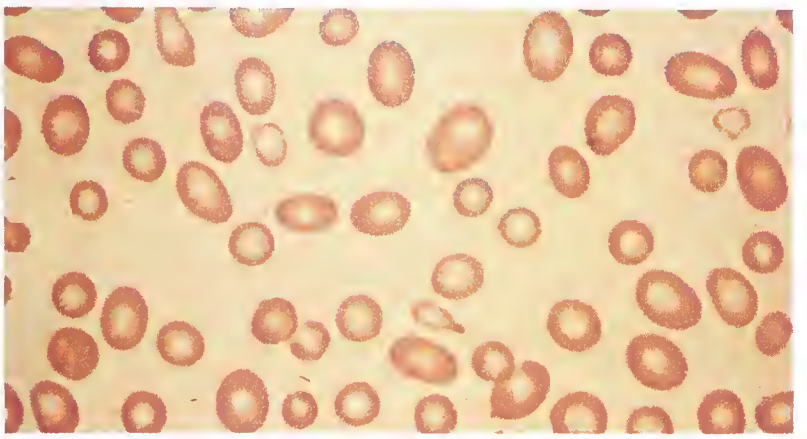

Figure 3a. *Macrocytosis, as in megaloblastic anemia. The red cells are large and oval-shaped.*

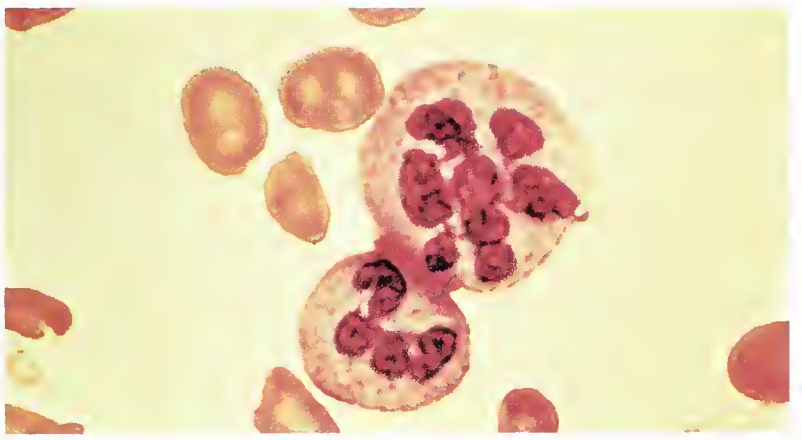

Figure 3b. *High power view of the peripheral film in megaloblastic anemia. Large oval macrocytes are themselves dwarfed by huge, hypersegmented neutrophils.*

Macrocytic anemia in which the MCV is only slightly elevated (100fL < MCV < 110fL) has a variety of causes and requires a thorough examination of the peripheral blood film to diagnose. This degree of MCV elevation is commonly seen in individuals whose erythrocytes form stacks called **rouleaux** on the peripheral blood film (e.g. in multiple myeloma; see Figure 4). The rouleaux are misinterpreted by the cell counter as large red cells, leading to a slight elevation of the MCV. This type of anemia may result from:

- Smoking or alcohol use
- Liver disease
- Hypothyroidism
- Marrow infiltration or marrow hypoplasia/aplasia

Additional investigations (e.g. serum TSH, liver function tests, bone marrow biopsy) may be necessary to definitively diagnose the cause of macrocytosis.

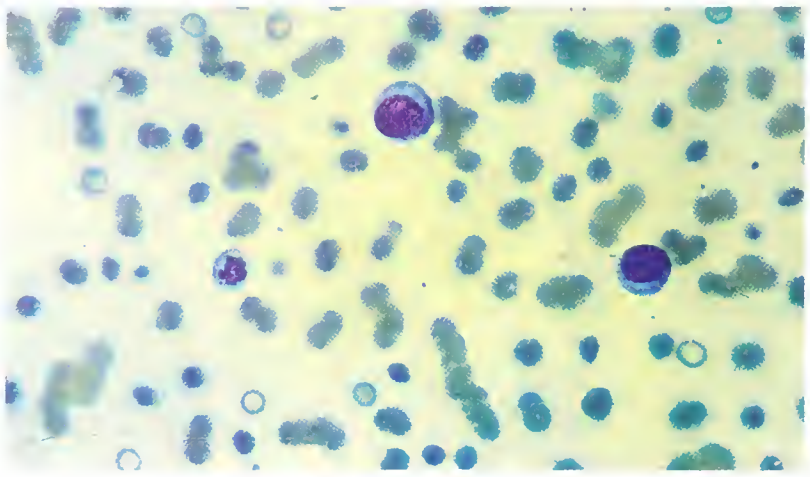

Figure 4. *Peripheral blood film showing rouleaux formation. In some areas, red cells are stacked together. In this case, the cause was multiple myeloma (two large myeloma cells appear on the film). However, rouleaux formation has many other causes.*

Low Hemoglobin and Normal MCV: Normocytic Anemia

Finally, there are many types of anemia in which the MCV is normal (between 80 and 100fL). These are the **normocytic anemias** (see Table 4).

The **anemia of chronic disease** arises in the setting of chronic neoplastic, inflammatory, infectious, renal, and endocrine diseases. The fundamental defect is an inability to remove iron from its stores in hepatocytes and cells of the reticuloendothelial system. Erythropoietin levels are inappropriately low for the degree of anemia. This type of anemia is normochromic and normocytic, but may be microcytic in severe cases. Usually, it resolves when the underlying disease process is treated.

Hemolytic anemia refers to a state in which there is accelerated destruction of red cells. There are two general mechanisms by which hemolysis occurs: 1) defects intrinsic to the red cells, leading to their destruction; 2) a process extrinsic to the red cells promoting their destruction. Hemolytic anemias can also be classified as either congenital or acquired. Table 5 summarizes these concepts.

Table 4
Normocytic Anemias

- Anemia of chronic disease
- Hemolytic anemias
- Renal failure
- Liver disease
- Endocrine disorders (hypothyroidism, hyperthyroidism, Addison's disease)
- Connective tissue diseases
- Primary bone marrow abnormalities
- Myelodysplasia
- Marrow infiltration
- Myelofibrosis
- Marrow aplasia

Clinically, hemolytic anemia is accompanied by the symptoms described in table 2, along with those of the underlying disease state (e.g. purpuric lesions) and sometimes, overt signs of hemolysis (jaundice and hemoglobinuria).

The various intrinsic causes of hemolytic anemia all result in a shortening of the red cell lifespan, which is normally between 90 and 120 days. This occurs either because the spleen recognizes the cells as faulty or because they are intrinsically unstable. In contrast, in the case of extrinsic processes, hemolysis occurs because of damage to otherwise normal red cells. Such processes include the binding of cytotoxic autoantibodies (in the various autoimmune hemolytic anemias), mechanical shearing across uneven surfaces (in thrombotic thrombocytopenic purpura and

Table 5
Types of Hemolytic Anemia

	Intrinsic RBC Defects	Extrinsic Processes
Hereditary	Hemoglobinopathies (e.g. sickle cell anemia) Hereditary spherocytosis Other RBC membrane protein defects Enzyme defects (G6PD deficiency, pyruvate kinase deficiency, etc.)	
Acquired	Paroxysmal nocturnal hemoglobinuria Spur cell anemia	Immune hemolysis Hypersplenism Microangiopathic hemolysis (TTP, DIC)* Pathogen toxins, etc.

**TTP = thrombotic thrombocytopenic purpura; DIC = disseminated intravascular coagulation*

disseminated intravascular coagulation) and excessive destruction due to an enlarged spleen (hypersplenism).

Spherocytosis refers to hemolytic anemia in which destruction results from the adoption of an abnormal spherical shape by the red cells. It may be the result of an inherited red cell structural defect (hereditary spherocytosis) or from an autoimmune (Coombs' test positive) hemolytic anemia. The spherocytes are readily apparent on the peripheral blood film (Figure 5).

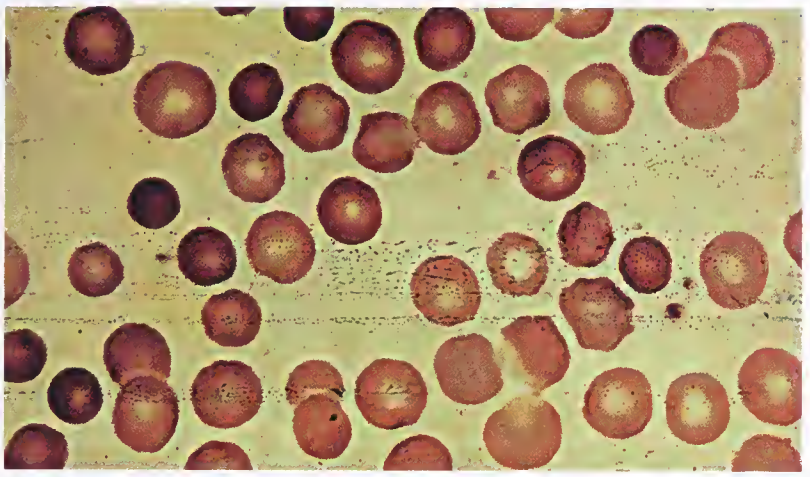

Figure 5. *Spherocytosis. Some red cells appear relatively normal, while others appear small and spherical. Spherocytosis produces a normocytic anemia because the MCV is not actually reduced: the cells only appear small on the peripheral film because they are spherical rather than disk-shaped. Since spherocytes are destroyed more rapidly than their normal counterparts, spherocytosis results in a hemolytic anemia.*

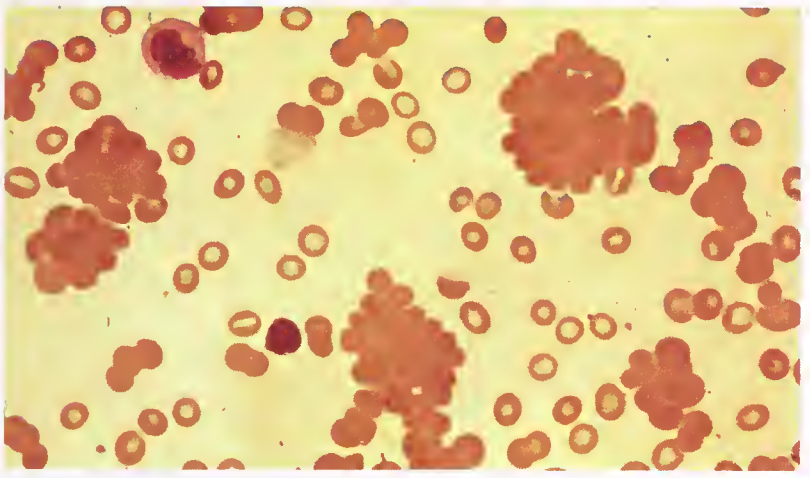

Figure 6. *Peripheral blood film from a patient with cold agglutination disease. Many red cells are agglutinated together in an IgM-mediated process.*

An example of an autoimmune hemolytic anemia is **cold agglutinin disease**, which is an IgM-mediated process. The peripheral blood film reveals **agglutination** of red cells (Figure 6), which is diagnostic. This condition requires emergency treatment.

Sickle cell anemia is a chronic hemolytic anemia resulting from an inherited hemoglobinopathy. A mutation in the (-globin gene, when present in the homozygous state, promotes adhesiveness between Hb molecules within the red cell, particularly at low oxygen tension (pO_2 < 80mmHg). When this occurs (owing to exercise, infection, etc.), the sickle hemoglobin polymerizes, comes out of solution, and distorts the red cell into the characteristic sickle shape. This leads to acute vaso-occlusive crises as the abnormal red cells become trapped in capillaries. **Sickle cell trait**, found in individuals heterozygous for the mutation, is mild and sickling occurs only at very low oxygen tension (pO_2 < 40mmHg).

In any normocytic anemia, it is especially helpful to examine the peripheral blood film, because this will often reveal the cause of the anemia. For instance, **schistocytes** or fragmented red cells are suggestive of microangiopathic hemolysis (Figure 7), while **burr cells** may indicate uremia. Sickle cells are diagnostic of sickle cell anemia (Figure 8) or sickle cell trait. An elevation in the number of reticulocytes (see below) suggests elevated marrow output in response to a hemolytic process; when they are not elevated, a non-hemolytic anemia is more likely.

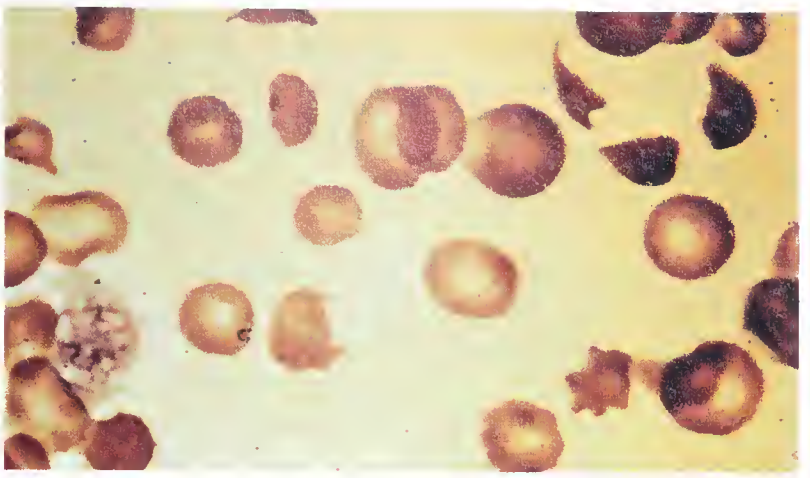

Figure 7. *Red cell fragments (schistocytes) on the peripheral blood film, as shown here, indicate that an angiopathic hemolytic process is occurring. This may be microangiopathic (DIC, TTP) or due to prosthetic heart valves and similar devices. Note that the red cells are irregular and appear to have been torn to pieces.*

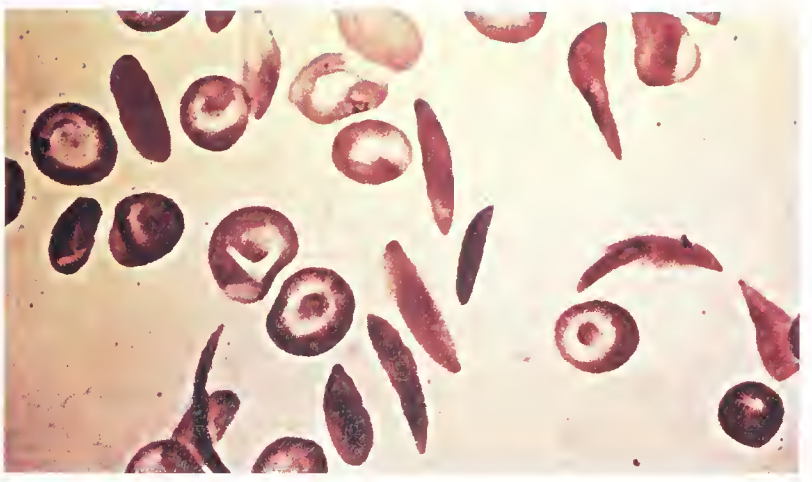

Figure 8. *Sickle cell anemia is a hemolytic anemia associated with characteristic sickled red cells.*

Importance of the Reticulocyte Count

Reticulocytes represent immature red cells that still possess ribosomes and messenger RNA, and are actively synthesizing globin chains. In normal individuals, a small number of reticulocytes can be found in the peripheral blood. Their numbers are increased, however, when red cell destruction or acute red cell loss (i.e. hemorrhage) is occurring or has just occurred. In such situations, the reticulocyte count is included with the CBC.

The reticulocyte count is reported in both relative and absolute terms. The relative reticulocyte count is a percentage of the total number of red cells accounted for by reticulocytes, while the absolute count is the number of reticulocytes per unit of blood

volume. The relative reticulocyte count considered on its own can be misleading, because changes in its value could represent changes in the actual number of reticulocytes, changes in the number of red cells, or both. Therefore in interpreting reticulocyte counts, the absolute number of reticulocytes must be considered.

High Hemoglobin: Polycythemia

When the Hb is elevated above the normal range, the RBC count is also abnormally high. This state is known as **polycythemia** and can result from decreased plasma volume (**spurious erythrocytosis,** a hemoconcentration effect, that may result from diuretic or alcohol use), an increase in red cell mass (true polycythemia), or a combination of the two. Special studies using radiolabelled red cells are needed to identify a true increase in red cell mass, as the hematocrit is not accurate for this purpose. The causes of true polycythemia may be classified as primary or secondary.

Primary polycythemia or **polycythemia rubra** vera is due to malignant transformation of a red cell precursor and is usually a relatively indolent disease.

Secondary polycythemia is driven by higher-than-normal erythropoietin production and can result from smoking, high-altitude living, chronic lung disease, and certain tumours (hepatoma, hypernephroma).

Polycythemia causes an increase in the viscosity of the blood (**hyperviscosity**). The symptoms and signs of polycythemia are consequences of hyperviscosity and prolonged blood flow times: angina, thrombosis, neurologic signs, and peripheral cyanosis.

Step 2. Consider White Blood Cells.

The white blood cell count (WBC) measures the total number of circulating leukocytes in peripheral blood and it is usually presented as the absolute value x 10^9/L. The total WBC count contains subsets of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Normal ranges of peripheral circulating white cells are found in Table 1. The primary function of leukocytes is to maintain host defenses against infection, especially bacterial infections. Monocytes and granulocytes ingest and kill the microorganisms, digest tissue debris, and release inflammatory mediators while lymphocytes activate and participate in the immune response. Alterations in the WBC count may represent a primary disorder of leukocyte production or may reflect a secondary response to specific disease processes.

Neutrophils

There are several pools of neutrophils in the body. In the bone marrow, there is a mitotic pool, a maturation pool, and a storage pool. In the peripheral circulation, there is a circulating pool and a marginating pool; the latter consists of neutrophils adherent to the vascular endothelium. Finally, there is a tissue pool consisting of extravasated neutrophils. Neutrophils move from the bone marrow to the circulating pool, then to the marginating pool, and finally into the tissue pool. They may also shift between the marginating and circulating pools. The peripheral neutrophil count, however, measures only the circulating pool. Hence, shifts of neutrophils between the circulating and marginating pools may cause an apparent abnormality of the peripheral neutrophil count.

Leukocytosis is the elevation of the WBC count greater than two standard deviations above the mean of one or more subsets of circulating white cells. The most common cause of leukocytosis is **neutrophilia**. Neutrophilia is defined to be the absolute neutrophil count (ANC) greater than 7.5 x 10^9/L. The most important acute cause of neutrophilia requiring prompt medical attention is infection. Neutrophilia is frequently observed in bacterial infection, inflammatory diseases, hemorrhage, and certain neoplasms. Some causes of neutrophilia are listed in Table 6. Neutrophilia can result from increased production of neutrophils as in the case of chronic infections and myeloproliferative disorders. It may also result from movement of neutrophils from the marrow storage or marginating pools to the circulating pools, which occurs in the acute inflammatory response. Finally, neutrophilia may be a consequence of chronic steroid therapy, which inhibits neutrophil egress from the circulating pool.

Table 6
Causes of Neutrophilia

Familial
Bacterial infections (acute or chronic)
Inflammation
Acute hemorrhage or hemolysis
Drugs
• Cytokine injection
• Corticosteroids
Malignancy
• Myeloproliferative disease
• Metastatic cancer
• Tumour necrosis

The leukemias are malignancies of myeloid and lymphoid precursor cells. **Acute myeloblastic leukemia (AML)** is the major acute leukemia in adults, while **acute lymphoblastic leukemia (ALL)** is the predominant acute leukemia of childhood. Both acute leukemias may be associated with a very high WBC count. This can be a medical emergency, because when the WBC count exceeds approximately 10^{11}/L, the patient is at risk of **leukostasis** (obstruction of small vessels by the leukemic blasts). AML and ALL can usually be distinguished on the peripheral blood film (see Figures 9 and 10). There are several other acute and chronic leukemias, and **chronic lymphocytic leukemia (CLL)** is depicted in Figure 11 for comparison.

Persistent neutrophilia of 3 x 10^{10} cells/L to 5 x 10^{10} cells/L above the normal range or greater is called a **leukemoid reaction**. This term is used to distinguish this degree of neutrophilia from leukemia since infections and some disease processes may cause an elevation in the white cell count resembling those seen in certain types of leukemia. For instance, pneumonia, other acute bacterial infections, burns, malignant disease, intoxications,

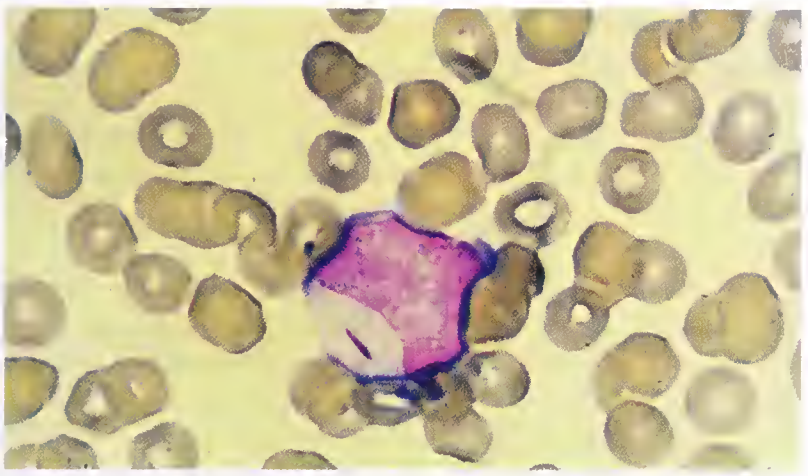

Figure 9. *This large leukemic blast contains a cytoplasmic inclusion called an Auer rod, which establishes the diagnosis of acute myeloblastic leukemia (AML). Compare this cell with the blast in Figure 10.*

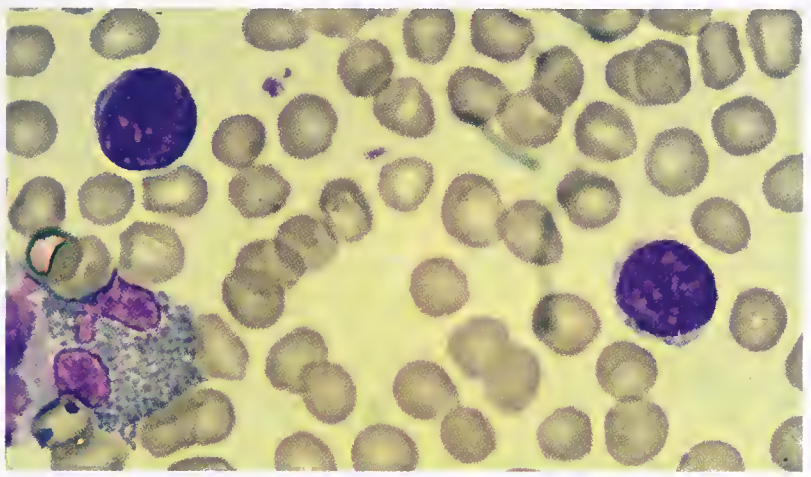

Figure 10. *This film contains a leukemic blast (at lower left) that, although still large, is smaller than the one in Figure 9 and has much less nuclear material. The patient has acute lymphoblastic leukemia (ALL).*

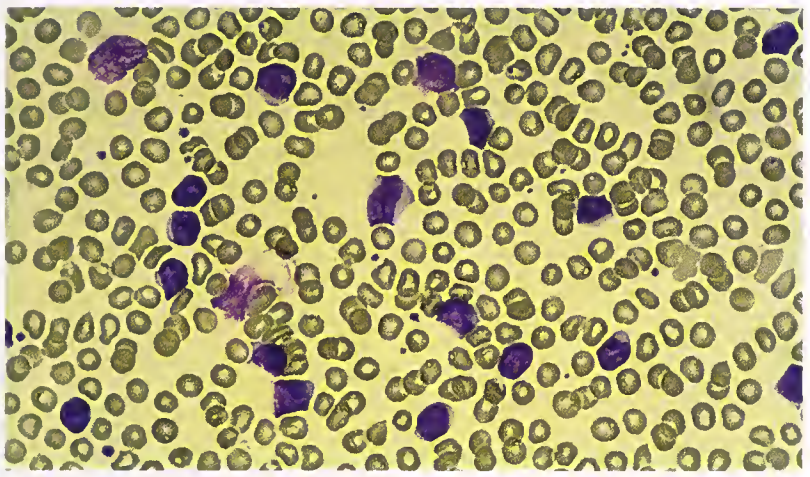

Figure 11. *In chronic lymphocytic leukemia (CLL), there is a leukocytosis consisting of abnormal small lymphocytes. Many examples are seen here. Note that some of the malignant cells have spilled their nuclear material onto the slide; these are called* ***smudge cells****.*

and severe hemorrhage can mimic myeloid leukemia. Other examples include lymphoid leukemia, which can be mimicked by pertussis, TB, and infectious mononucleosis, as well as monocytic leukemia, which can also be mimicked by TB. In addition, one distinguishing feature is that in a leukemoid reaction, the circulating neutrophils are usually mature and not clonally derived.

Neutropenia is defined as an absolute neutrophil count of less than 2.5 x 10^9/L. An ANC of less than 1 x 10^9/L is worrisome, while a count below 0.5 x 10^9/L is considered life threatening because of the risk of losing of control over endogenous microbial flora. When there are fewer than 0.2 x 10^9 neutrophils/L of blood, the inflammatory response is absent. Neutropenia can result from depressed production of leukocytes such as that seen in aplastic anemia, ineffective mitosis such as in megaloblastic anemia, increased peripheral destruction seen in hypersplenism, increased marginal pooling in viremia, or accelerated loss from tissues. The causes underlying neutropenia are summarized in Table 7. Some clinical manifestations seen in neutropenia include fever, chills, infection by opportunistic organisms, and septicemia in its later stages. Evaluation of neutropenia involves a search for an underlying disease, examination of the bone marrow to rule out marrow failure, and elimination of drugs.

Table 7
Causes of Neutropenia

Decreased production
- Stem cell disorder
- Congenital
- Acquired
- Aplastic anemia
- Leukemia
- Myelodysplasia
- Viral
- Vitamin deficiency
- Specific neutrophil suppression
- Congenital
- Acquired
- Drug
- Immune suppression

Increased destruction in the periphery
- Overwhelming infection
- Immune destruction
- Drugs (e.g. ionizing radiation, chemotherapy)
- Related to collagen-vascular disease
- Idiopathic
- Isoimmune disease (newborn)

Increased pooling with destruction
- Splenomegaly and hypersplenism
- Complement activation
- Pseudoneutropenia

Lymphocytes

The lymphocyte count in peripheral blood is increased (**lymphocytosis**) in viral infections and it is more commonly elevated in viral than in bacterial infections. These infections include Epstein-Barr virus (EBV), cytomegalovirus (CMV), and rubella. Lymphocytosis is also seen in toxoplasmosis, pertussis, brucellosis, and chronic lymphocytic leukemia. In pertussis, a lymphocyte count of 8 x 10^{10}/L may be observed. The underlying cellular mechanisms that cause the increased lymphocyte count during an infection remain unknown; however, the interactions among the microorganism, the cellular and humoral immune systems, as well as various immune modulators have been implicated. Furthermore, many patients who develop a leukemoid reaction due to an infection may also present with lymphocytosis. Atypical lymphocytosis with large, immature-looking cells is characteristic of infectious mononucleosis, caused by EBV (Figure 12). Other viruses that cause atypical lymphocytosis include CMV, hepatitis, mumps, varicella, herpes, influenza, and roseola infantum. Patients presenting with symptoms of a viral

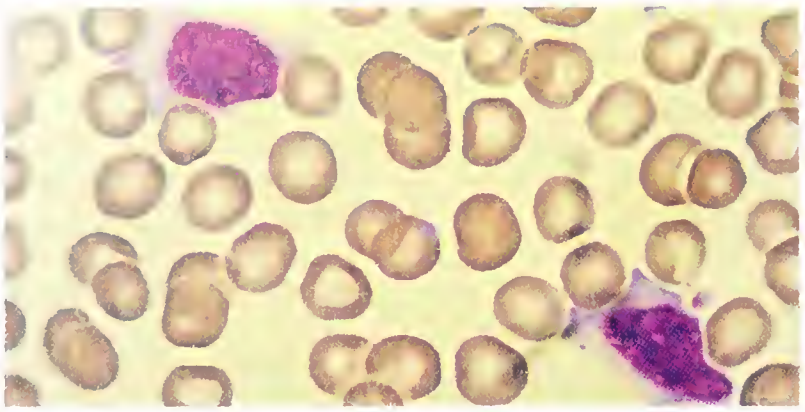

Figure 12. *Platelet satellitism. Owing to a reaction of the patient's platelets with the anticoagulant EDTA in the blood collection tube, the platelet count is artificially low and the peripheral blood film reveals platelets adherent to white cells as "satellites."*

infection with mild lymphocytosis that does not resolve within four to six weeks should be investigated for the possibility of chronic lymphocytic leukemia.

Lymphocytopenia is often seen with the administration of steroid therapy, systemic lupus erythematosus, uremia, and Legionnaire's disease. This condition is also observed in HIV patients as well as patients requiring chemotherapy and radiotherapy. Moreover, many acute bacterial infections cause mild lymphocytopenia in the range of less than 1 x 10^9 lymphocytes/L.

Monocytes

Monocytes are circulating mononuclear leukocytes, which can migrate into tissue to become macrophages. Monocytes function as phagocytes at sites of inflammation and participate in the killing of intracellular microorganisms. Monocytes have three basic functions: secretion of inflammatory mediators and cytokines, ingestion of microorganisms and debris, and interaction with lymphocytes, a process essential to the immune response.

Monocytosis, an increase in the number of circulating monocytes in the peripheral blood, is associated with certain infections such as tuberculosis, brucellosis, subacute bacterial endocarditis, malaria, and Rocky Mountain spotted fever. This condition is also seen in malignancies, myeloproliferative disorders, hemolytic anemias, and chronic idiopathic neutropenias. Patients with collagen vascular diseases, sarcoidosis, and ulcerative colitis may also develop an associated monocytosis.

Monocytopenia occurs with acute infections, stress, and following glucocorticoid administration. In addition, monocytopenia is observed in aplastic anemia, hairy cell leukemia, and acute myelognous leukemia. This condition can also result as a consequence of myelotoxic and immunosuppressive drug therapy.

Eosinophils

Eosinophils participate in many immune responses by secreting lipid mediators, interacting with lymphocytes, and inducing release of inflammatory mediators from mast cells. Primary disorders of eosinophils are rare; more typically, these cells fluctuate in response to the systemic influences mediated by infection and inflammation. **Eosinophilia** is the presence of greater than 0.5 x 10^9 eosinophils/L of blood. An increased eosinophil count may signify a parasitic infection, usually a helminthic infection. Other possible causes of a raised eosinophil count include allergic reactions to drugs such as iodides, aspirin, sulfonamides, penicillins, and cephalosporins. Allergies such as hay fever, asthma, serum sickness, allergic vasculitis, and eczema are also commonly associated with eosinophilia. Furthermore, eosinophilia is seen in other underlying disorders such as collagen vascular diseases (e.g. rheumatoid arthritis, eosinophilic fasciitis) and malignancies (e.g. Hodgkin's disease, chronic myelogenous leukemia, cancer of lung, stomach, pancreas, ovary or uterus).

Eosinopenia occurs with stress such as in the setting of an acute bacterial infection or following administration of glucocorticoids. This condition is relatively rare.

Basophils

The normal functions of basophils are not completely understood. **Basophils** have been primarily identified with allergic reactions and some delayed cutaneous hypersensitivity states. Basophilia, an increase in basophil count in the peripheral blood, is seen in association with immediate hypersensitivity reactions, iron deficiency, chronic renal failure, lung carcinoma, and the anemia of chronic disease. This condition may also be present in inflammatory and infectious states such as ulcerative colitis, rheumatoid arthritis, influenza, and tuberculosis. Basophilia may also occur in myeloproliferative disorders.

Basopenia refers to a basophil count lower than normal. Like cosinopenia, this condition is extremely rare.

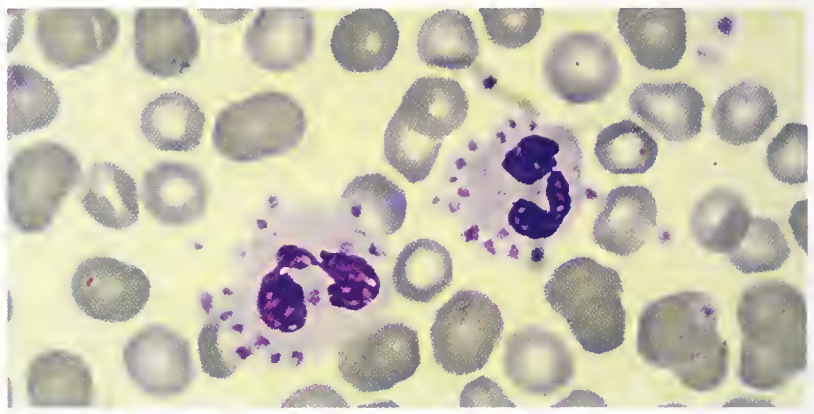

Figure 13. *The "spreading" lymphocytes that appear to intercalate between the red cells on this peripheral blood film are diagnostic of infectious mononucleosis.*

Step 3. Consider Platelets.

Quantitative platelet disorders include thrombocytopenia and thrombocytosis. **Thrombocytopenia** is defined as an absolute decrease in the platelet count below 150 x 10^9/L. In general, hemorrhagic complications develop as the platelet count decreases. Platelet counts between 40 to 60 x 10^9/L may be associated with an increased risk of post-traumatic bleeding, where as a platelet count less than 20 x 10^9/L increases the risk of spontaneous bleeding. The mechanisms underlying thrombocytopenia include decreased platelet production, increased platelet destruction, sequestration, increased consumption, and intravascular dilution. In addition, an artifactual cause of thrombocytopenia results from an interaction between the patient's platelets and the anticoagulant EDTA once the blood has been collected. This reaction occurs in about 1 in 2000 patients. Exposure of the platelets to EDTA causes platelet aggregation. Furthermore, the platelets often adhere to white cells, a phenomenon called **platelet satellitism**. The cell counter counts these aggregates as a single platelet or ignores them, leading to

very low platelet counts. An examination of the peripheral blood film (Figure 13) assures the clinician that the patient does not truly have thrombocytopenia. Table 8 summarizes the mechanisms of thrombocytopenia with some representative examples of each. Some causes of thrombocytopenia will be discussed in the following section.

Heparin-Induced Thrombocytopenia (HIT)

In contrast to the other drug-induced thrombocytopenias, heparin-induced thrombocytopenia can result in life-threatening thromboembolic events. **HIT-1** is a non-immune process in which the decrease in platelet count is usually seen early in the course of heparin therapy. HIT-1 is a transient phenomenon, and the platelet count will return to normal once the heparin is discontinued. There is no intravascular thrombosis as this condition is likely due to platelet aggregation and sequestration. Heparin can also result in an immune-mediated thrombocytopenia, **HIT-2**, by forming an immunogenic complex with platelet factor 4. This condition usually begins at between five and fifteen days of heparin therapy. The typical platelet count in patients with HIT-2 ranges from 25 to 100 x 10^9/L; however, it is generally agreed that a fall in the platelet count by one-third of the baseline value following the initiation of heparin therapy should be considered potentially significant. Clinical complications of HIT-2 include serious bleeding, intravascular thrombosis, heparin-induced skin necrosis, mesenteric artery or vein occlusion, and acute platelet activation syndromes.

Idiopathic (Autoimmune) Thrombocytopenic Purpura (ITP)

ITP is an immune-mediated destruction of platelets that occur in the absence of drug or toxin exposure. There is increased platelet destruction due to an autoimmune process in this condition. Patients with ITP have been found to have an abnormally high level of IgG on their platelet surfaces. In the circulation, platelet survival is decreased, with the IgG-coated platelets being cleared in the spleen. ITP can occur at any age and is clinically associated with the development of petechiae, ecchymoses, mucosal bleeding, and epistaxis. Another common presentation of ITP is following a viral infection. Severe complications include GI bleeding and intracranial hemorrhage and these tend to occur when the platelet count is below 10 x 10^9/L.

Thrombocytosis is defined as an increase of the platelet count above 450 x 10^9/L. This condition can occur in three forms: physiologic or transitory, reactive or secondary, and primary or essential thrombocytosis. A **physiologic thrombocytosis** may be observed after stress or exercise. This represents the mobilization of preformed platelets from the spleen or lung due to the influence of epinephrine. A **reactive thrombocytosis** occurs as a result of the increase in platelet production in response to hemorrhage, hemolysis, certain infections, iron deficiency, inflammatory diseases, splenectomy, or malignancy. No hemorrhagic or thrombotic complications are associated with reactive thrombocytosis, thus therapy to reduce the platelet count directly is not necessary. **Primary (essential) thrombocytosis** is a myeloproliferative disorder arising from neoplastic transformation of a pluripotent stem cell. Some clinical features seen in this condition include an elevated platelet count, splenomegaly, platelet dysfunction, and an increased risk of hemorrhagic and thromboembolic events. Morphologically, giant platelets may be present in the circulation.

Conclusion

The CBC is a commonly ordered investigation that is included in many diagnostic work-ups. It provides a glimpse into some systemic functions as well as selective disease processes. The thorough examination and interpretation of the CBC will provide an invaluable source of information in the management of a number of different diseases. The systematic approach presented here may serve as a guide to help organize the information obtained every time a CBC is encountered.

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Table 8
Causes of Thrombocytopenia

Mechanism	Examples
Decreased platelet production	• Drugs (ethanol, anticonvulsants) • Toxins • Viral infections (varicella, mumps, HIV, EBV, CMV, parvo) • Nutritional defects (vitamin B12, folate, or iron deficiency) • Myelophthisic processes • Radiation or systemic chemotherapy • Ineffective production
Increased platelet destruction	• Drug-induced thrombocytopenia (quinidine, sulfas, thiazides, heparin) • Alloimmune thrombocytopenia (post-transfusion purpura, neonatal alloimmune thrombocytopenia, platelet refractoriness) • Autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, HIV)
Increased platelet consumption	• Thrombotic thrombocytopenic purpura • Hemolytic uremic syndrome • Disseminated intravascular coagulation
Sequestration	• Splenomegaly
Dilutional	• Massive transfusion with stored blood

Renal Cancer: Misfortune Strikes Twice!

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Case Presentation

A 71-year-old man with a 30-year history of renal calculi and microscopic hematuria presented to his family doctor with gross painless hematuria and flank pain of one-week duration.

Abdominal ultrasound revealed three masses suggestive of neoplasia in the right kidney: a large mass (9.6 cm diameter) in the lower pole, and two smaller masses (2.5 cm and 1.5 cm) in the lower pole.

The patient was referred to a Urology clinic at a university-affiliated teaching hospital.

Past medical history revealed infrequent but recurring renal calculi with pain and gross hematuria for approximately 30 years in addition to a 7-year history of low-grade proteinuria and borderline elevated serum creatinine. Four years prior to this presentation, the patient had been placed on sodium bicarbonate to prevent the precipitation of further renal stones. One month ago, the patient experienced urinary frequency and burning consistent with a urinary tract infection (UTI). In two previous abdominal ultrasounds (1961 and 1991) while no renal masses observed, the kidneys were noted to be small in size with an irregular cortex, possibly suggestive of a prior inflammatory condition in the kidneys.

Other comorbities include a 15-year history of hypertension and hyperlipidemia (both of which are well controlled with medications), prostatism, and chronic back pain resulting from a work-related back injury.

What differential diagnoses would explain these clinical findings?

Differential Diagnosis

Gross painless hematuria. Hematuria can be described as either gross (visible to observer) or microscopic (only visible by microscopic exam). Clinically speaking, the measurement of >1-2 RBC's per high power field (considered normal) is considered hematuria. Furthermore, bloody urine of clinicopathological significance (true hematuria) must always be differentiated from pseudohematuria. The latter may be caused by a number of substances which produce a red-brown discoloration of the urine such as dyes (beets, rhodamine), proteins (hemoglobin, myoglobin), certain drugs (rifampin, coumadin, aspirin/NSAIDs, pyridium and laxatives with phenolphthalein), and menses in the female.[1,2]

Once the presenting hematuria has been identified as true hematuria, there are various means of causal classification including patient age, source of the blood or the timing of the perceived blood in relation to the urinary stream (refer to Tables 1, 2 and 3).

Table 1
Classification of Hematuria by Age

Age (years)	Possible Cause
0 –20	GMN, UTI, congenital abnormality
20 – 40	UTI, stones, bladder tumour
40 – 60	male: bladder tumour, stones, UTI female, UTI, stones, bladder tumour
> 60	(BPH), bladder tumour, UTI

Table 2
Classification of Hematuria by Source

Source	Possible Cause
Pre-renal	• throughout urinary stream: anticoagulants, coagulation defects, sickle cell disease, leukemia
Renal	• throughout urinary stream: renal cell carcinoma, transitional cell carcinoma, Wilm's tumour, pyelonephritis, TB, glomerulonephritis, trauma, stone, infarct, polycystic kidneys, AVM
Ureter	
Bladder	• stone, tumour
Urethra	• cystitis, tumour, stone, polyps
	• urethritis, stone, tumour, stricture

Table 3
Classification of Hematuria by Timing

Timing	Possible Cause
Initial blood in initial portion of urinary stream	• lesion in the anterior urethra
Terminal blood in terminal portion of urinary stream	• lesion at the level of the bladder neck/trigone
Total blood throughout urinary stream	• lesion at the level of the bladder or above

Generally speaking, painless hematuria is usually due to renal, vesicular or prostatic disease. Kidney or bladder tumours must always be considered in the evaluation of an asymptomatic patient with painless hematuria (particularly gross hematuria); other causes may include renal calculi or cysts, sickle cell disease, hydronephrosis, IgA nephropathy and polycystic disease. The presence of urinary RBC casts is virtually pathognomonic of glomerulonephritis.[1,2]

Given this patient's past history of renal problems, a pattern of gross painless hematuria and without RBC casts, the presence of right flank pain, and the radiographic findings of several renal masses, a diagnosis of renal tumour was highly suggested and investigated further.

The consulting urologist requested that the patient undergo a chest X-ray (CXR), bone scan and computed tomography (CT) to further investigate the suspected diagnosis and the possibility of metastases. The CT scan confirmed the presence of a dense mass in the lower pole of the right kidney with possible invasion of the tumour into the liver. The CXR and the bone scan did not show evidence of distant metastases to lung or bones.

The patient was scheduled to undergo a right radical nephrectomy for removal of a neoplastic kidney.

Clinical Diagnosis: Renal cell carcinoma.

Surgery

One month later, the patient underwent a radical nephrectomy of the right kidney. There were no complications with the surgery and the post-operative course was uneventful, with the exception of a low-grade fever 2 days following his operation. He was discharged one week following his surgery, and was informed that he would need to return every 6 months to check for the occurrence of lung metastases.

The removed right kidney (measuring 15 cm x 12 cm x 8.5 cm) was submitted *in toto* for fixation in 10% neutral buffered formalin. A bulging extracapsular tumour (1.3cm) at the anterior mid-portion of the kidney was noted on gross observation. A second, much larger tumour occupied the entire width of the lower pole of the kidney. Measuring 8.5 cm at its widest dimension, this extensive growth appeared yellowish-tan in colour, and partly hemorrhagic.

Fixed sections were embedded in paraffin and 5 µm sections were cut, mounted on glass slides, and stained with hematoxylin and eosin. Light microscopy of the tumour sections revealed extensive areas of round cells containing clear cytoplasm and some nuclear atypia, a finding consistent with clear cell renal carcinoma. The tumour had invaded into perinephric adipose tissue, but there was no involvement of the hilar vessels.

Pathological Diagnosis: Renal clear cell carcinoma of the right kidney, grade 2, stage pT3.

One month post-operatively: The patient returned for follow-up of his renal function by the urologist. Laboratory analysis of his serum revealed the following abnormalities that are listed in Table 4.

Table 4
Blood Serological Values One Month Post Right Radical Nephrectomy

	Value	Range
Serum Creatinine	182 µmol/L	60-120 µmol/L
Hemoglobin	115 g/L	126-180 g/L
Hematocrit	0.35	0.42-0.52
WBC	8.2 x 10^9/L	4-11 x 10^9/L
WBC Differential	Unremarkable, except for eosinophils = 0.5 x 10^9/L	0.0 – 0.4 x 10^9/L

Although the creatinine was elevated, this was not a concern at the time since it was expected that a transition period would be required before the remaining kidney took over all renal function. The patient was prescribed $FeSO_4$ since it was thought he had microcytic anemia, a common condition in patients with chronic renal failure.

Over the next 5 months: A CXR did not demonstrate any evidence of lung metastases. However, the patient's serum creatinine was noted to be elevated on several occasions (178 µmol/L, 203 µmol/L (N: 60-120 µmol/L), which indicated a referral to a nephrologist for investigation of remaining renal function. Prior to this visit, the patient had begun to experience difficulty with occasional incomplete voiding and with the initiation and termination of his urinary stream. On inquiry, the patient denied experiencing any edema post-operatively.

The physical examination by the nephologist revealed that the patient was alert, oriented, and in no obvious discomfort. Vital signs were within normal limits and the patient was not in any respiratory distress. Chest examination revealed normal audible breath sounds throughout all lung fields. Precordial examination revealed normal first and second heart sounds, and a quiet systolic ejection murmur. There was mild edema on the dorsum of the left foot (20-year history since a fracture of the left leg) and no edema in the right leg. The abdomen was examined to rule out an incisional hernia that may have developed post-operatively. There were no masses or pulsations, no tenderness or rebound, and no hernias or other abnormalities in the RLQ. There were no observable signs of volume overload or uremia (such as pericardial rub or distended jugular veins).

Medications included: mevacor 20 mg p.o. OD, viskazide 10/25 OD, alopurinol 300 mg p.o. OD, surgam 300 mg p.o. and cytotec 200 mg p.o. PRN for back pain. The patient has no known medication allergies.

Urinalysis revealed moderate proteinuria (1 g/L with trace hemolyzed blood) while microscopy of the spun urine sediment showed microscopic urine hematuria. A recent 24-hour urine collection had also demonstrated the presence of proteinuria (2.5 g/24 hour).

In summary, this is a patient in chronic renal failure that requires special monitoring to slow further deterioration of his renal function.

Clinical Plan: Management for this patient included close monitoring: (1) to determine whether the functional status of the remaining kidney was stable, improving or declining; and (2) to follow the possible emergence of disease development in the remaining left kidney. The patient was advised that he was in good health for his annual fall trip to Florida and given medical clearance to travel.

Table 5 illustrates abnormalities found upon further investigations of the patient between seven months to one-year following his right radical nephrectomy.

Table 5
Investigations 7 months to 1 year following Right Radical Nephrectomy

INVESTIGATION	VALUE/ FINDING	RANGE
Hemoglobin	103 g/L	126-180 g/L
Serum creatinine	196 µmol/L	60-120 µmol/L
Urine creatinine	11 µmol/L	7-16 µmol/L
Proteinuria	2.15 g/L	<0.15 g/day
Chest X-RAY	Negative for lung metastases	
Abdominal Ultrasound	Fatty liver with no radiographic evidence of hepatic metastases; One solid mass (2.3 cm x 1.5 cm x 1.8 cm) at the mid-to-lower poles of the left kidney.	
Enhanced and unenhanced CT scan with contiguous axial imaging (to further investigate renal mass seen on ultrasound)	An ill-defined, low-attenuating structure which enhanced following the administration of radiographic contrast material was identified in the intrapolar region of the left kidney (corresponded to the solid mass seen on U/S); Precontrast tissue showed 30 Hounsfield while post-contrast density was measured at 62 Hounsfield units (indicating the structure was probably vasular in nature); 1 cm mass superior to the upper pole of the left kidney; left renal contour was noted to be lobulated, left renal vein was patent and there was no evidence of intraluminal filling defect within the inferior vena cava at the level of the renal veins	
Abdominal angiogram of left renal artery	Mass in the upper and middle third lobes of the left kidney, and evidence of irregular small tumour-like vessels in the same area; no tumour extension seen outside of the left kidney	

Abdominal ultrasound, CT scan of the remaining kidney, and abdominal angiogram demonstrated the unfortunate tumour involvement of the patient's remaining kidney. Considering the patient's recent renal history, a pre-operative clinical diagnosis of renal cell carcinoma was made.

Clinical Diagnosis: Renal cell carcinoma of left (remaining) kidney.

The investigations and diagnosis were discussed with the patient. The patient was presented with two options: (1) He could undergo another radical nephrectomy of his remaining kidney. The risks of this second operation, including infection, bleeding, and the requirement of lifetime dialysis were explained to the patient; (2) His second option was to forego the nephrectomy, accept the status quo and live without dialysis. It was explained to him that optimal treatment involved the installation of a hemodialysis access site followed by radical nephrectomy of his remaining kidney. After consultation with his two daughters, the patient agreed to this advised plan of management. The patient was referred to the end stage renal disease clinic for education and guidance on diet adjustment.

Surgery: The vascular surgeon created an arterio-venous fistula for the dialysis access site. Two weeks later, a left radical nephrectomy was performed.

The tumour was located close to the hilum; therefore a nephron-sparing partial nephrectomy was not possible. The dimensions of the removed left kidney were 18 cm x 9 cm x 6 cm. The left adrenal gland was left behind. Post-operatively, the patient was noted to have a foul-smelling, yellow urethral discharge, intense urgency to micturate 2-3 times per day, and chills. These symptoms were attributed to pyocytis, a collection of desquamated cells in the bladder irritating the patient. There was no bleeding or fever. Treatment with the antibiotic nitrofurantoin was begun, and the symptoms resolved.

The tumour in the left kidney was revealed to be a giant renal medullary interstitial cell tumour at stage pT3. It extended focally beyond the renal capsule into the perirenal adipose tissue. Immunohistochemistry was negative for RCC, S100, SM-actin, AE1-AE3 vimentin, myoglobin, Hmb45, and desmin.

There is a high risk of recurrence with this type of renal carcinoma, with eighty-five percent of these tumours recurring during the first three post-nephrectomy years. Therefore, transplantation is not an option for this patient until at least three years post-operatively.

Nephrology was consulted to begin hemodialysis post-operatively and to assess his food and electrolyte status.

Second Pathological Diagnosis: Giant renal medullary interstitial cell tumour; stage pT3.

Renal Cancer

Introduction. Tumours of the kidney encompass a diverse spectrum of both benign and malignant varieties. While benign kidney tumours are incidental findings at autopsy and generally of little clinical significance, most solid kidney tumours are malignant and of much importance. An estimated 95,000 deaths worldwide per year are accounted for by kidney cancer. Most commonly (yet incorrectly) referred to as renal cell carcinoma, kidney cancer is more prevalent in men than in woman (3:2), and in Canada it is the 6th most common cancer, with a lifetime probability risk of 1.5%.[3] The median age at diagnosis is 65, although children can occasionally present with renal cancer. The most common subtype, renal cell carcinomas (RCC) account for 85-95% of malignant neoplasms arising from the kidney and represent about 2% of cancer deaths in adults.[4-6]

Epidemiology. Most renal cancer is sporadic; however, a number of genetic and environmental risk factors have been identified. Smoking is the most prominent risk factor (two-fold incidence). While additional identified risk factors include obesity, hypertension, having a first-degree relative with kidney cancer, and exposure to asbestos, petroleum products, and heavy metals,[7,8] certain unusual variants of autosomal dominant familial cancers have been identified which predispose younger individuals to the development of renal cancer. For example, up to two-thirds of patients with von Hippel-Lindau (VHL) disease develop renal cysts and multiple renal cell carcinomas.[4,6] VHL is a rare familial disease in which tumours develop in a number of locations in the affected individual. Both copies of the VHL gene, which is linked to chromosome 3p, are inactivated in the tissue of all VHL-associated kidney cancers. Furthermore, gene therapy with replacement wild type VHL suppresses the *in vitro* growth of kidney-cancer cells. Other hereditary renal cancers include familial clear cell carcinoma and hereditary papillary carcinoma.[6]

Clinical Presentation and Diagnosis. The classic triad for kidney cancer (particularly RCC) is flank/back pain, hematuria (gross or microscopic), and a palpable flank mass.[4-6, 9-11] Any one of these symptoms may present independently as an early indicator of tumour presence. Unfortunately, few patients (10%) present with this symptom complex which reliably indicates advanced disease.[9-11] Hematuria is the most common presenting sign, followed by pain, palpable mass and fever of unknown origin. Other common presenting symptoms include hypertension, anemia, weight loss and fatigue. Paraneoplastic symptoms are frequently associated with these malignancies, and may even represent the presenting symptoms in some patients with renal cancer. The most common syndromes are erythrocytosis, hypercalcemia, nonmetastatic hepatic dysfunction (Staufer syndrome) and hypertension.[6,10] A more detailed list of possible features of renal cell cancer is presented in Table 6. Increasingly, asymptomatic kidney cancers are incidentally diagnosed by radiography.[12]

A typical plan for the evaluation of a patient with a renal mass will include investigation by renal U/S, CT scan and/or renal arteriograms. Features indicative of renal neoplasm seen by these investigations are solid vs. cystic mass, internal echos (U/S), vascularity and contrast enhancement. U/S or IVU confirm the presence of a renal mass, while CT provides sufficient staging information (such as density, local extension and nodal and venous involvement) for definitive surgical management.[10-12]

Table 6
Simplified Classification of Renal Tumours[10]

Benign tumours
- Renal capsule
- Renal parenchyma
- Vascular tumours
- Cystic lesions, dysplasia, hydronephrosis
- True oncocytoma

Tumours of Renal Pelvis
- Benign papilloma
- Transitional cell carcinoma
- Squamous cell carcinoma
- Adenocarcinoma

Pararenal tumours
- Benign vs. malignant

Embryonic Tumours
- Nephroblastoma (Wilms')
- Embryonic, mesotheliomatous tumours
- Sarcomas

Nephrocarcinoma
- Renal cell carcinoma (aka adenocarcinoma, hypernephroma)
- Papillary cystadenocarcinoma

Other malignancies
- Primary: mesenchymal, hemangiopericytoma, myeloma
- Secondary: metastatic lesions

Classification. Various classifications have been used over the years by pathologists to describe the broad spectrum of renal tumours. Table 7 describes the most common and important subdivisions such as benign tumours, tumours of the renal pelvis, pararenal tumours, embryonic tumours, nephrocarcinoma and other malignancies.[10] The following discussion will focus on the 2 renal cancer subtypes described in this patient: renal cell carcinoma (RCC) and giant renal medullary interstitial cell tumour.

Table 7
Common Presenting Symptoms in Patients with Renal Cell Carcinoma[2,4,5,9,10]

Asymptomatic
Classic triad
Gross or microscopic hematuria
Pain
Palpable mass
Weight loss, cachexia
Fever
Varicocele
Hypertension
Erythrocytosis
Hypercalcemia
Raised ESR
Abnormal liver function; raised AP (nonmetastatic hepatic dysfunction; Staufer syndrome)
Neuromyopathy
Amyloidosis
Acquired dysfibrinogenemia

Renal Cell Carcinoma

While 5-10% of primary renal tumours are urothelial (occur in the renal pelvis), the majority of malignant renal tumours are determined to be renal cell carcinomas (RCC; 85-95%) of proximal tubule origin.[4-6, 9-11] Also known as renal cell adenocarcinoma or hypernephroma, RCC most commonly affects the upper poles although it may arise throughout the kidney. RCC is characterized by a tendancy to invade the renal veins and grow as a solid column of cells inside the renal vessels which frequently extends to the inferior vena cava and possibly as far as the right heart.[6,9,10,13] This patient was monitored frequently by CXR and no invasion of the vascular system was detected.

Clear cell carcinoma. RCC can be further categorized on the basis of predominant cell type. The major types of RCC are clear cell (nonpapillary), chromophilic (papillary), chromophobic, oncocytic and collecting duct or Bellini duct renal carcinomas.[5,10] Each of these subtypes of RCC is characterized by different genetic and morphological features and varying prognoses.

Comprising 70-80% of all RCC, clear cell carcinoma is characterized by clear or granular cytoplasm. This abundant RCC subtype usually occurs as solitary, unilateral, spherical masses 3-15 cm in diameter containing rounded or polygonal cells.[6,10,11] On gross observation, there may be large areas of yellow-grey-white necrosis that distorts the renal outline, however, the tumour margins typically remain sharply defined and confined within the renal capsule. A varying growth pattern may sometimes be discerned, such that areas with tumour involvement display either solid, trabecular, tubular or even cystic patterns of growth. Under light microscopy, most tumours of clear cell morphology are described as well-differentiated, although there may be interspersed areas of nuclear atypia, bizarre nuclei and giant cells.[6,10]

While most clear cell carcinomas arise sporadically, there is also a discernible pattern of heredity and association with VHL.[4-6, 9-11] The most prominent genetic abnormality (98%) is deletion or unbalanced translocation of chromosome 3 (t3;6, t3;8, and t3;11).6,10 Disturbance of the region of chromosome 3 at 3p14 -3p26 results in either loss or hypermethylation of the gene for VHL and a greatly elevated risk of RCC development.[6,13] Deletions of the 3p gene (VHL) are not associated with any other RCC subtype.[6]

The remaining subtypes of RCC arise much less frequently than clear cell carcinoma: chromophilic (10-15%), chromophobic (5%), oncocytomas (3%) and collecting duct or Bellini duct carcinoma (2%).5 Each RCC subtype is characterized by distinct genetic associations, specific histological features and variable growth patterns.

Renomedullary Interstitial Cell cCncer

The second *de novo* cancer of this patient's remaining kidney was of a highly unusual and extremely rare type known as a giant renal medullary interstitial cell tumour. Also known as medullary fibroma, these tumours have the ultrastructural features of renal interstitial cells with no malignant propensities.[6,10] Mostly occurring in women, these uniformly benign tumours are usually found at autopsy as small foci of firm grey-white tissue. Typically identified in the pyramids of the kidneys and less than

1 cm in diameter, renomedullary interstitial cell tumours are composed of collagenous tissue and fibroblast-like cells.[6,10] Due to their benign nature, partial nephrectomy is usually adequate, however a radical nephrectomy is frequently performed because of diagnostic uncertainty.

Staging and Prognosis. CT allows the differentiation between tumours that are locally confined, and those that are locally advanced. CT findings are congruent with the pathological staging in 84-91% of tumours.[14] The stages and associated prognosis of renal cell carcinoma are summarized in Table 8.

Table 8
Stages and Prognosis of Renal Cell Carcinoma[5, 10]

STAGE	EXTENT OF TUMOUR GROWTH	PROGNOSIS AT 5 YEARS
T1	Small tumour, minimal caliceal distortion, confined to renal capsule	66%
T2	Large tumour, caliceal distortion, confined to renal capsule	64%
T3a	Tumour extension to perirenal fat or ipsilateral adrenal gland (confined by Gerota's fascia)	60%
T3b	Renal vein involvement	
T3c	Renal vein and vena cava involvement	
T4b	Vena cava involvement above diaphragm	
N1	Single ipsilateral node involved	
N2	Multiple regional, contralateral, or bilateral nodes involved	20%
N3N4	Fixed regional nodes	
T4a	Juxtaregional nodes involved	11%
	Spread to contiguous organs except ipsilateral adrenal gland	
M1	Distant Metastases	<11%

Common metastatic sites for renal cell carcinoma are lung, bone, adrenal, and brain. Hematogenic metastases are ruled out by CXR. Liver, adrenal gland, and the contralateral kidney can be assessed by CT scan. A bone scan is only indicated in symptomatic patients or in cases of elevated alkaline phosphatase or elevated calcium. Likewise, brain metastases must only be ruled out in symptomatic patients.[15]

At diagnosis 20% of patients have disseminated disease; 25% have locally advanced disease. Approximately one-third of all patients with tumour limited to kidney at time of diagnosis will subsequently develop metastasis post-operatively.[16]

Management of Renal Cell Carcinoma.

For locally confined tumours. The standard treatment for stage pT1 and pT2 tumours is radical nephrectomy. This procedure involves en bloc removal of the kidney, the fatty capsule, and the adrenal gland, as well as primary control of the renal vessels. There is some controversy in the literature as to whether adrenectomy is necessary since leaving the adrenal gland behind has not affected prognosis for cases in which the tumours involved the lower two-thirds of the kidney, and a CT scan of the adrenal gland was normal.[17] A partial nephrectomy or nephron-sparing surgery can be performed in selected patients depending upon the size and location of the tumour, especially in those individuals with bilateral tumours. The results of tumour excision equal those of nephrectomy if the tumour size does not exceed 4 cm in diameter.[15] However, it is important to remove all of the tumour, and in cases of doubt, biopsies should be taken from the tumour bed.[18] Lymph node dissection is also controversial. For stage pT3b, T3c, T4b, the tumours should be resected from the renal vein or vena cava.[5]

For advanced disease. Advanced renal cell carcinoma encompasses patients with: (1) hematogenic metastases at the time of diagnosis; (2) lymph nodes metastases at the time of diagnosis; (3) local recurrence, lymph node or hematogenic metastases in the follow-up of potential curative treatment.[15] Metastatic renal cell carcinoma is associated with a dismal survival, and there is no curative therapy.

Research has been active in attempting to discover a treatment(s) to improve the prognoses of patients with metastatic renal cell carcinoma. Several options that have been explored include hormonal therapy, chemotherapy, nephrectomy, and immunotherapy.

Two options that have been proven not to be useful are hormonal therapy and chemotherapy. The response rate to progestins is very low at 1-2% and do not last.[18] Unlike other solid malignancies, cytotoxic chemotherapy is not the standard of treatment for renal cell carcinoma. This is due to the expression of the multi-drug resistance product p-glycoprotein that actively effluxes drug out of the tumour cells. Response rates are less than 20% for even the most "effective" chemotherapy agents.[5]

This leaves the following treatment modalities, all of which are currently the subject of intense exploration in the scientific and medical community. Treatment options in metastatic patients include: (1) nephrectomy alone; (2) combination immunotherapy and nephrectomy; or (3) immunotherapy alone.

The evidence is at best fair for the role of nephrectomy alone in primary metastasized renal cell carcinoma. In selected patients with pain, recurrent urinary hemorrhage or flank pain, nephrectomy may help alleviate some of the tumour complications to improve the quality of life, despite the fact that the natural history of the disease is not altered. However, spontaneous regression of metastases following surgery of the primary tumour is very low at 0.001-0.8%.[19] The nephrectomy-only approach is complicated by the fact that metastases are usually in multiple sites and invade more than one organ. Favourable predictors of survival after nephrectomy include single organ of first recurrence, curative resection of first metastases, long disease free interval (>12 months), solitary site of first metastases, and metachronous presentation with recurrence.[20] These conditions are seen only in a small minority of patients. Table 9 demonstrates some of the advantages and disadvantages of surgery for metastatic renal cell carcinoma.

Immunotherapy includes the use of biological agents such as interferon-α or interleukin-2 (IL-2), and lymphokine-activated killer cells. Clinical trials with these agents used alone, or in combination (i.e. recombinant IL-2 plus interferon-α; recombinant

IL-2 plus lymphokine-activated killer cells) were given as intravenous bolus or continuous IV infusions. Toxicities from such agents include a capillary leakage syndrome, fever, chills, fatigue, and hypotension.5 Interferon-(is postulated to act either through its known anti-proliferative activity on tumours or its ability to stimulate host mononuclear cells and upregulate major histocompatibility complex (MHC). In a study of 1042 patients, interferon-(produced response rates of 12%, but researchers selected for patients with good performance status, prior nephrectomy, long disease-free survival and lung predominant disease.[20] It is difficult to comment on the efficacy of immunotherapy alone because most studies of these biological agents were performed using patients following a nephrectomy. In addition, response rates ranged from 2-30%, with only a small minority experiencing long-term remissions.[22]

More randomized clinical trials are needed to answer questions such as which patients can tolerate immunotherapy post-operatively, and whether the removal of primary tumour increases the likelihood of an objective response to immunotherapy at the metastatic site.[16]

Directions for treatment in the more distant future include gene therapy, monoclonal antibody therapy, vaccine approaches, and the exploitation of dendritic cells in a kidney cancer vaccine. Gene therapy include the in vitro transformation of autologous tumour with cytokine genes, or by transferring cytokine genes into autologous T cells. Monoclonal antibodies that bind to tumour specific antigen, and deliver cytotoxic agents to tumour are already used therapeutically in colon cancer and non-Hodgkin's lymphoma. Renal cell carcinoma specific antigen G250 has been identified, and anti-G250 monoclonal antibodies have been found to react with 85% of all renal cell carcinoma cell lines, all clear cell renal cell carcinomas, and most non clear cell types, but not with normal kidney cells.[21] Current tests are underway to see whether these antibodies can be used in a kidney cancer vacine. Dendritic cells are antigen presenting cells, and these can be pulsed with tumour specific antigen to increase the stimulatory effect of T cell dependent immune response.

Lung metastases. Lungs are by far the most common site for renal cell carcinoma metastasis. The largest study (n=96) to date showed that complete excision of lung metastases resulted in 46% survival at five years for solitary lung metastasis versus 27% at five years for multiple lung metastasis.[23] Fischer and Schmid (1999) commented that "these days, it is probable that the majority of patients with pulmonary metastasis are treated with a combined concept of surgical excision of the primary tumour and subsequent systemic IL-2 therapy."[24]

Bone metastases. Metastasis to the bone occurs in <10% of renal cell carcinomas. The role of surgery is mainly palliative.[25] The literature reports a functional improvement and pain reduction in 80% of patients, but this was associated with a 20% complication rate from secondary amputations of the extremities.[24] Palliative radiotherapy is indicated for symptomatic bone metastases with a 30-40% survival rate at 5 years for solitary bone metastases.[15]

Liver metastases. Autopsies show that when liver metastases occurs, there is a >98% chance that metastases has occurred in other organs as well.[24] In cases of liver metastasis or cases where the patient's general physical condition is poor, surgical procedures are generally not recommended.

Observation alone. In 10% of patients with metastatic disease, there was no progression in disease for more than 12 months. Due to the uncertain responses to systemic therapy, and the associated side effects from treatment, one option for asymptomatic patients with metastases is close observation until disease progression or symptoms occur.[5]

Patient Outcome

Four months post-operatively (following second nephrectomy): Abdominal and pelvic ultrasound showed no evidence of metastasis. The liver, pancreas, spleen, and gallbladder were normal. There was some atherosclerotic change in the aorta, but there were no masses seen in either renal bed. It was recommended that follow-up chest x-rays be done every six months to rule out any lung metastases.

Six months post-operatively: Chest x–ray revealed no lung metastases at this time, and the patient appeared to be doing well on hemodialysis. Soon after the chest x-ray, the patient presented with symptoms similar to the pyocytis following his second nephrectomy. These included intense urgency 2-3 times per day, foul smelling urine, and yellow discharge, but no fever. He

Table 9
Surgery for Metastatic Renal Cell Carcinoma[16]

ADVANTAGES	DISADVANTAGES
• Treatment/prevention of tumour complications (hematuria, bleeding, flank pain)	• Treatment alternatives available (immobilization/irradiation)
• Spontaneous regression of metastases	• Waste of time
• Remove source of new metastases	• Dangerous (perioperative morbidity/mortality)
• Reduction of tumour burden	• No survival benefit ever proven
• Removes trap for trafficking lymphocytes	• May compromise immune system
• Psychology/Quality of life?	• Psychology/Quality of life?

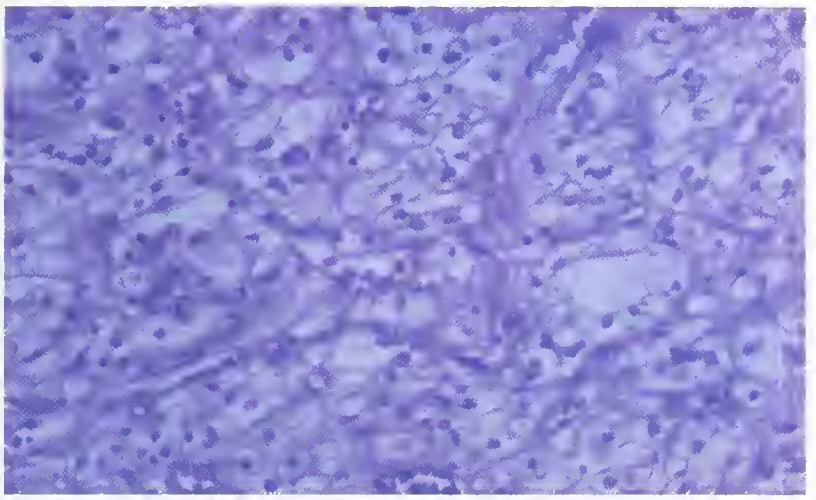

Figure 1. *Haematoxylin and eosin stain of renal cell carcinoma.*

was treated with nitrofurantoin, and the symptoms again resolved. In addition, he experienced pain in his left lower back and parathesia for 2-3 hours following the x-ray. This pain was intermittent, worsened with movement, and improved with rest. Investigations with a bladder wash and a cystoscopy were unremarkable.

One year post-operatively: Aside from the urethral discharge which was improving, the patient was asymptomatic. Unfortunately the chest x-ray revealed a suspicious 2.5 cm nodule in his left lower lung. He was booked for a spiral CT to further investigate the nodule. The following ominous signs were observed: enlarged subcarinal nodes (30 mm x 25 mm), multiple small lymph nodes in the left axilla, and significant nodules located throughout the right and left lung fields, the largest being in the left (25 mm x 24 mm). Based on the patient's lifetime history as a non-smoker, unremarkable pulmonary history, and excellent physical condition, the nodules were more likely to be metastases from his renal carcinoma rather than a primary bronchogenic carcinoma in his left lower lobe. A fine needle aspirate was performed to biopsy the nodule in the lower lobe of the lung. Since the mediastinoscopy was positive for metastatic kidney cancer, a thoracotomy was not an option because there were multiple lymph nodes scattered in the mediastinum.

The patient remains on hemodialysis Mondays, Wednesdays, and Fridays, and is asymptomatic regarding his pulmonary metastasis. However he is extremely depressed regarding his condition. Given the aggressiveness of his kidney tumours, and the diffuse and rapid metastatic spread to his lungs, his prognosis is poor. Medical oncology follow-up was arranged.

Acknowledgements

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Case Reports from the University of Toronto Faculty of Medicine

Stephen D. Milone, M.Sc. (0T2) and Manish Sood, B.Sc. (0T2)

Case 1

Stephen D. Milone, M.Sc.
Second Year Medical Student, University of Toronto, the members of the 1999 Moscati Mission to Bolivia (Mission Coordinator Luigi A. Castagna M.D., F.R.C.P.(C)), and the staff at Hospital Andres Cuschieri, Colcapirhua-Cochabamba, Bolivia

A 10 year old boy presented to the members of the mission in Chapare, Bolivia with a fixed, rock-hard lesion on the right side of his face (Figure 1.1-1.3). He was otherwise well and, although in no discomfort, had been somewhat ostracized by his peers due to his disfigurement. Two years previously, he had suffered a fall that caused a contusion over the right maxilla and a persistent hematoma. The rock hard lesion soon became apparent and got progressively larger over that time frame.

What is the differential diagnosis?
What investigations should be ordered?

FIGURE 1.1. *Photo of gross features.*

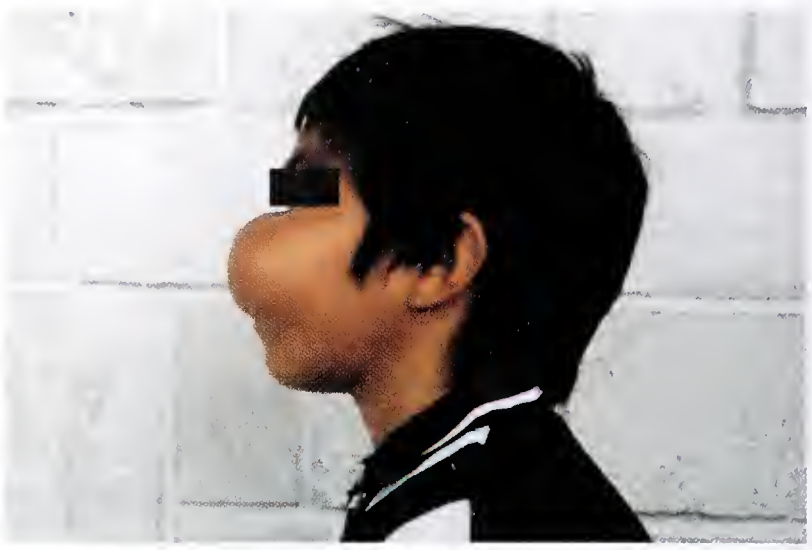

FIGURE 1.2. *Photo of gross features.*

FIGURE 1.3. *Photo of gross features.*

Case 2

Michael Bayek, M.D.
Division of Internal Medicine, Toronto General Hospital

A 75 year old male with a significant history of chronic alcohol abuse was admitted to hospital for the work up of a brief loss of consciousness that resulted in a car accident. He sustained only minor head trauma. Past history was significant only for two previous craniotomies, one for an injury with a nail gun 50 years ago, and the second resulting from a motorcycle accident in the mid 1970's. Within 24 hours of his admission, he developed increasing confusion, agitation, tremor and hallucinations. Following a negative CT scan (Figure 2.1), a diagnosis of alcohol withdrawal was made and he was treated with diazepam. Over the next 24-48 hours he became increasingly drowsy presumably due to the diazepam. He developed an aspiration pneumonia and hyponatremia, and was treated with antibiotics and water restriction respectively. His level of consciousness improved, but not back to his previous baseline. He then developed difficulties with speech and swallowing.

Without further imaging or medical information, what is the most likely diagnosis?

FIGURE 2.1. *CT scan of the head.*

Case 3

Jay Keystone, M.D. and Martha Fulford, M.D.
Divisions of General Internal Medicine and Infectious Diseases, Toronto General Hospital

A 60 year old male travels to Belize for a two week vacation in both urban and rural areas. One week after his return, he notes boils on his arm (Figure 3.1 and 3.2), which occasionally produce sharp, stabbing pains.

What is the differential diagnosis?

FIGURE 3.1. *Gross features of the patient's boils.*

FIGURE 3.2. *Gross features of the patient's boils.*

Case 4

Michael Bayek, M.D.
Division of Internal Medicine, Toronto General Hospital
Hillar Vellend, M.D., F.R.C.P. (C)
Department of Medicine, Mount Sinai Hospital

A 31 year old, male presents to the emergency room at Mount Sinai Hospital with a 5 day history of rash on the neck and trunk, increasing abdominal pain, fever, malaise, and cough with increasing shortness of breath. He was previously well apart from a past history of severe psoriasis treated with methotrexate 5 mg q week. He is heterosexual and in a monogamous relationship. There is no previous history of blood transfusions or IV drug use. There are no occupational exposures. He is a smoker and occasionally drinks alcohol. On examination, blood pressure is 140/80 mmHg, heart rate 120 beats/min, respiratory rate 30/min, and he is febrile at 39.5°C. He is in mild respiratory distress with crackles bilaterally. There is no evidence of CHF or murmurs. He is fully alert and oriented. Neurological examination is normal. Examination of the skin reveals raised, maculo-papular lesions over the neck, arms and upper trunk (Figure 4.1).

What is the diagnosis?
What is the etiology of his lung findings

FIGURE 4.1. *Skin rash over the upper trunk and arms.*

Case 5

Dan Penisko, M.D., F.R.C.P.(C)
Division of Internal Medicine, University Health Network, Toronto Western Hospital

A 71 year-old male presents with retrosternal chest pain. The pain originated four months prior, presenting only upon exertion and was relieved with nitroglycerin. However, it has subsequently increased in severity and now presents without exertion. There is a 27 pack-year smoking history and an unknown cholesterol status. Physical examination reveals a heart rate of 100 bpm, a blood pressure of 134/64 supine and a JVP at the sternal angle. The apex is normal and upon auscultation, a grade 3/6, holosystolic murmur is heard. The murmur is maximally heard at the apex with the patient in left lateral decubitus and it radiates to the right 2nd intercostal and the left sternal border.

What is the diagnosis?

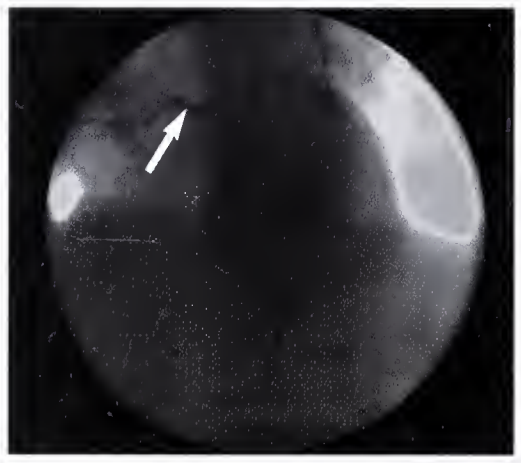

FIGURE 5.1. *Coronary angiography.*

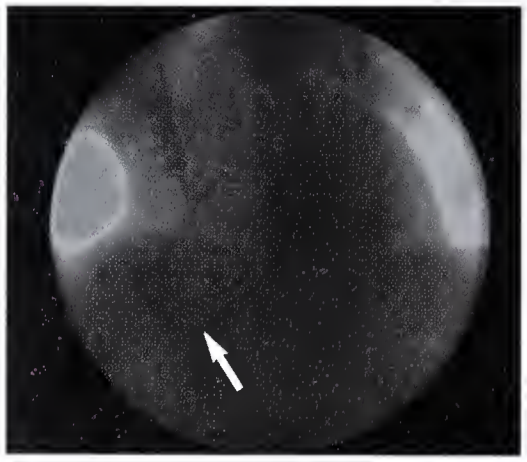

FIGURE 5.2. *Coronary angiography.*

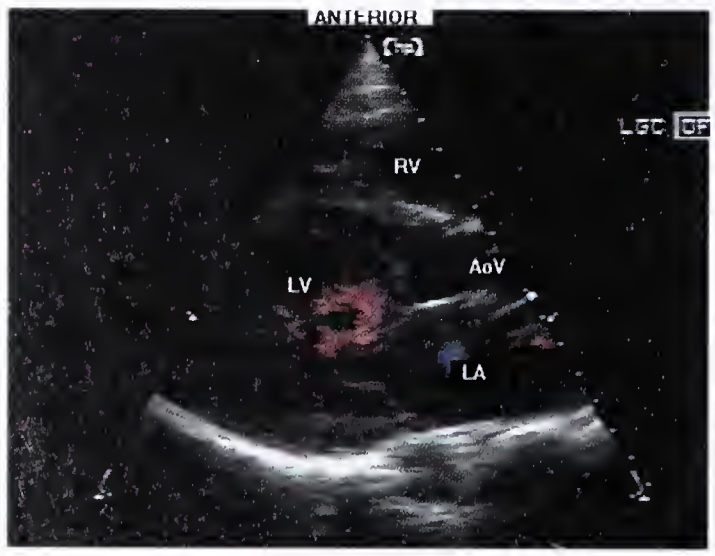

FIGURE 5.3. *Transesophageal echocardiography with Doppler.*

Case 6

Dan Penisko, M.D., F.R.C.P.(C)
Division of Internal Medicine, University Health Network, Toronto Western Hospital

A 58 year-old women presents with a five day history of difficulty swallowing during which she continually vomited undigested food. History is difficult to obtain due to a severe developmental delay. Physical exam reveals inspiratory stridor and decreased air to the lung bases bilaterally. There is no tracheal deviation or drooling. There is significant ulnar deviation of the digits bilaterally, with subluxation of the MCP joints and bilateral knee effusions. The abdominal exam reveals splenomegaly. Laboratory investigations reveal marked neutropenia. A tracheostomy was performed due to progressive respiratory compromise.

What is the connection between the tracheostomy and the appearance of the hand?

What is the diagnosis?

FIGURE 6.1. *Photograph of hand*

FIGURE 6.2. *Photograph of tracheostomy*

Case 7

Dan Penisko, M.D., F.R.C.P.(C)
Division of Internal Medicine, University Health Network, Toronto Western Hospital

A 83 year old female was admitted to the hospital with a progressive shortness of breath for the last month. The history of the present illness included orthopnea, paroxysmal nocturnal dyspnea, ankle edema and pre-syncope. She is a non-smoker with a 10 year history of controlled hypertension, type 2 diabetes and a strong family history of cardiac illness. Her blood pressure was 190/95 sitting with a heart rate of 102 bpm and an elevated jugular venous pressure. Inspiratory crackles, dullness to percussion and decreased breath sounds were elucidated at the lower right chest. Cardiac auscultation reveled an S4 and there was peripheral, pitting edema from the knee distally. Carotid pulses revealed a bisfiriens pulse pattern. An initial chest X-ray was performed (see Figure 7.1).

What is causing the shortness of breath?
What is the most likely underlying etiology?

FIGURE 7.1.
Chest X-ray.

She was treated appropriately and five days later the symptoms returned along with a pulsus paradoxus of 13 mmHg and a recurrent elevated jugular venous pressure.

What is the diagnosis?

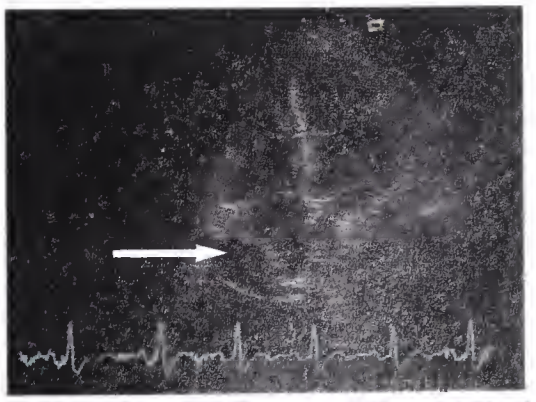

FIGURE 7.2.
Transesophageal echocardiography.

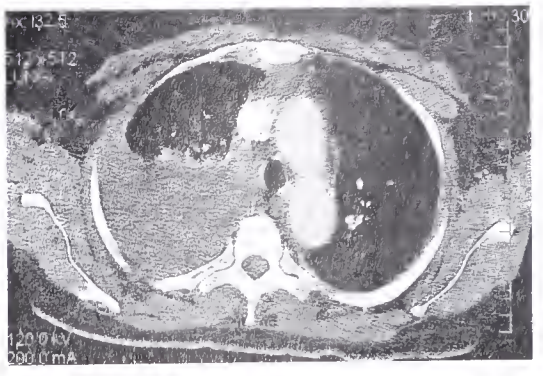

FIGURE 7.3.
MRI chest.

Case 8

W. Montanera, M.D., F.R.C.P.(C)
Division of Neuroradiology, University Health Network, Toronto Western Hospital

A 42 year-old male presents with a traumatic fall resulting in a fracture of the fibula. Upon questioning, it is discovered that the fall occurred due to a 'visual disturbance.' The patient describes a gradual vision loss, along with headaches, polyuria and polydipsia. Physical exam findings include mild papilledema and a right temporal hemianopsia.

What is the diagnosis?

FIGURE 8.1.
Cranial MRI with contrast.

Case 9

Kiran Madala, M.D., F.R.C.P.(C)
Clinical Associate in Hepatology, University Health Network, Toronto Western Hospital

A 77-year-old Italian male presents with fever, abdominal right upper quadrant pain, jaundice and pruritus. Previous travel history includes residence in Africa 30 years ago. Family history reveals a niece with Crohn's disease. On physical examination, he is jaundiced with some mild tenderness in the epigastric area. Laboratory results show AST = 66, ALT = 46, ALP = 343 and Total Bilirubin = 23.

What is the diagnosis?

FIGURE 9.
Endoscopic retrograde cholangiopancreatography (ERCP).

Case 10

Michael Bayek, M.D.
Division of Internal Medicine, Toronto General Hospital

An 83 year old previously well female presented to the emergency room with a 12 hour history of bloody diarrhea and crampy abdominal pain. There is no previous history of diarrhea and no recent travel or use of antibiotics. One day earlier, she had eaten fish and chips at a restaurant. Six days earlier, her daughter had had similar symptoms. On examination, blood pressure was 130/70 mmHg, heart rate 90 beats/min, respiratory rate 22 /min, and she was afebrile. She had mild diffuse abdominal tenderness. She was oriented with no neurological deficits. There was no rash and there were no active joints. In hospital, she was treated with ciprofloxacin. However, over the next several days her condition deteriorated with increasing confusion and shortness of breath. She became oliguric and laboratory parameters steadily worsened (Table 10).

What is the most likely diagnosis?

Table 10
Laboratory Investigations During Hospital Course

Day in Hospital	Hemoglobin	Platelets	Creatinine
1	152	206	117
2	171	239	123
3	148	195	151
4	136	123	194
5	114	90	224

And the diagnosis is....

Case 1

Diagnosis: Fibrous Dysplasia of the right superior maxilla

There was no invasion by the lesion into the surrounding tissues as shown by the CT (Figure 1.4). The lesion was biopsied and histological analysis revealed a proliferation of fibroblasts within a dense collagenous matrix that was widespread with occasional, benign, multinucleated giant cells. The histology was consistent with fibrous dysplasia. A hemimaxillectomy resection en bloque was performed by Dr. Ana Maria Gil in Colcapirhua-Cochabamba, Bolivia (Figure 1.5). Pathological analysis of the specimen revealed a 7 x 5 x 5 centimeter lesion consisting of multiple, irregular, bony fragments occupying approximately 25 cc of tissue (Figure 1.6). The boy is currently doing well medically (Figure 1.7).

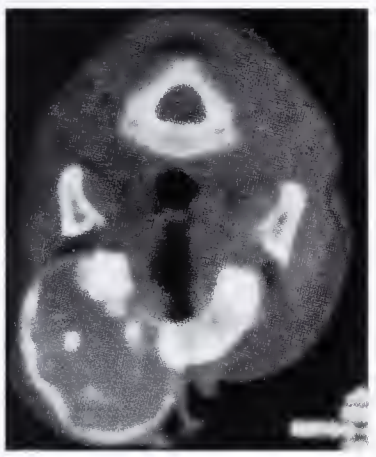

FIGURE 1.4. *CT scan of the head.*

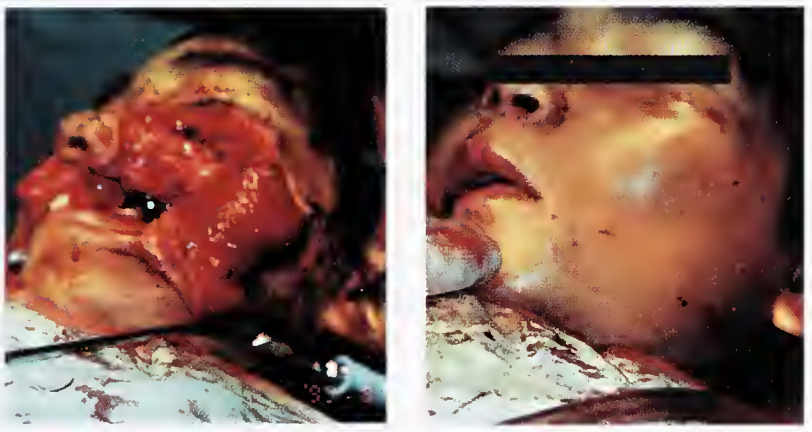

FIGURE 1.5. *Intra-operative photos of the hemimaxillectomy.*

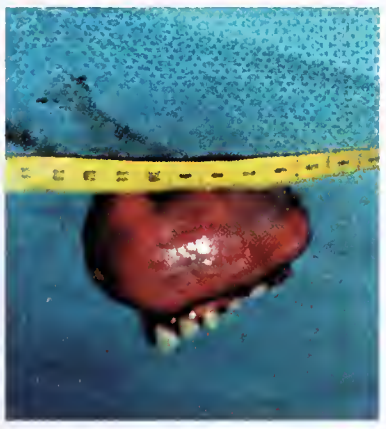

FIGURE 1.6. *Surgical specimen; resected right maxilla.*

FIGURE 1.7. *Patient, post-operatively.*

Case 2

Diagnosis: Chronic Subdural Hematomas.

There were no other focal neurological deficits on exam. Because of these problems a second CT scan was performed 11 days after his admission (Figure 2.2) which revealed chronic subdural hematomas. He improved on his own and since there were no other focal neurological deficits, he was treated conservatively and was successfully discharged home 1 month after his admission.

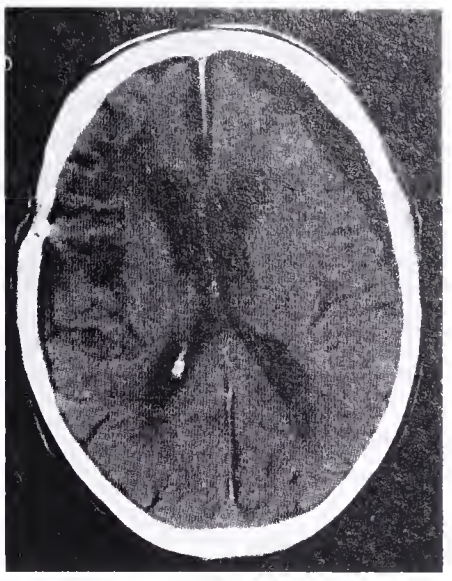

FIGURE 2.2. *CT scan of the head, 11 days after hospital admission.*

Case 3

Diagnosis: Myiasis

Myiasis, from the Greek myia for fly, is the infestation of vertebrates by fly larvae. This is a case of myiasis caused by the botfly, *Dermatobia hominis*, which is endemic to the forested areas of Central and South America. The female adult botfly captures a mosquito, or other blood-sucking arthropod, mid-flight and attaches her eggs to its abdomen. When the egg-carrying arthropod next takes a blood meal from a vertebrate, the eggs hatch in response to the increase in temperature. The hatched larva (or larvae) burrows into the subcutaneous tissues of the vertebrate host where it remains for 6-12 weeks. After this, it emerges, drops to the ground, and pupates into an adult fly. A small, usually pruritic papule forms at the site of entry which increases in size as the larva grows (Figure 3.1). It resembles a large furuncle or boil with a central punctum for a breathing hole. The anterior portion of the larva possesses two oral hooks that grasp and tear the flesh of the host upon which it feeds. This may result in intermittent deep pain. There may be drainage of serosanguinous fluid. The lesion itself is not usually tender. It may be possible to see larval movement or bubbles through the punctum.

Treatment consists of removal of the entire larva (Figure 3.3). One option is to simply allow the larva to mature and drop out naturally; however, this is unacceptable to the patient. Options for removal include asphyxiation or surgical removal, preferably the former. If it is not removed, it may die and be resorbed by the host. Asphyxiation involves occlusion of the breathing hole which forces the larva to move upward in search of air at which point it can be grasped by forceps and gently removed. Substances that have been used for the occlusion of the breathing hole include Vasoline, mineral oil, bacon fat, raw meat, butter,

chewing gum, crazy glue, and bees wax. As a last resort, surgical excision may also be attempted. It is important not to cut the larva as this may result in an intense inflammatory response in the host. Once the larva has been removed, no further treatment is required.

For another case report of furuncular myiasis, please refer to Lemon MA and Aeling JL. (2000). *NEJM.* 314:937.

FIGURE 3.3. *Resected larva*

Case 4

Diagnosis: Varicella Pnuemonitis

The patient also gave a history of recent exposure to a child with chickenpox. He was admitted with the presumptive diagnosis of varicella pneumonitis (Figure 4.2) and treated with IV Acyclovir. However, within 24 hours of admission, he suffered progressive respiratory deterioration: marked respiratory distress, respiratory rate 70 /min, heart rate 140 beats/min, blood pressure 140/90 mmHg. The patient was intubated and ventilated with high FiO2, PEEP, and neuromuscular blockade. On the 4th hospital day, the patient had a brief asystolic cardiac arrest due to mucous plugging. On neurological examination, there was spontaneous breathing and blinking to loud noise but he had positive doll's eyes, small pupils, roving gaze, no spontaneous movements, and upgoing toes bilaterally. A tracheosotomy was performed as well as a head-CT and an MRI. During the next 9 months, the patient remained in hospital and developed several nosocomial complications. There was no significant neurological recovery and he was ultimately transferred to a chronic care facility.

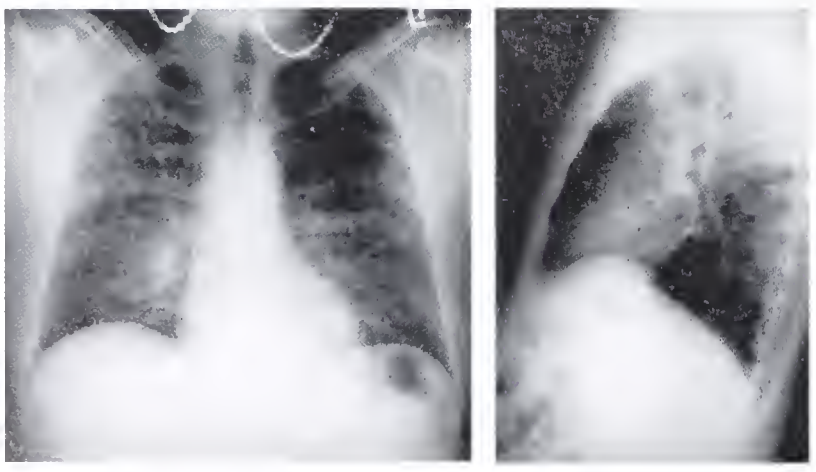

FIGURE 4.2. *Chest x-rays.*

Case 5

Diagnosis: Unstable angina due to diffuse coronary artery disease and severe mitral valve regurgitation

Figure 5.1 is a left anterior oblique view showing narrowing of the lumen of the left coronary artery. Significant narrowing is observed in the left main, circumflex and anterior descending coronary arteries. Figure 5.2 is a left anterior oblique view showing narrowing of the right coronary and the posterior interventricular arteries. The patient has significant triple vessel disease resulting in transient episodes of myocardial ischaemia. The echocardiogram with Doppler (Figure 5.3) depicts severe mitral regurgitation due to mitral valve prolapse with a subsequent ejection fraction of less then 20% (a Grade 4 left ventricle). Clinical detection of mitral regurgitation is accomplished through the auscultation of a holosystolic, high pitched murmur heard best at the apex in left lateral decubitus.

Case 6

Diagnosis: Felty's Syndrome

Felty's syndrome consists of chronic rheumatoid arthritis, neutropenia and splenomegaly. It is most common in individuals with long-standing disease who have high titres of rheumatoid factor and other systemic signs of illness. Figure 6.1 shows subluxation of the metacarpophalangeal joints and ulnar deviation of the digits, a characteristic hand deformity in rheumatoid arthritis. Chronic inflammation of synovial joints occurs in rheumatoid arthritis and, in this case, involved the articular surfaces of the cricoarytenoid joints in the larynx. This led to paralysis of the vocal cords along with the subsequent dysphagia and respiratory distress that required a tracheostomy.

Case 7

Diagnosis: Cardiac tamponade due to metastases

Figure 7.1 shows a large pleural effusion in the lower right lung that was felt to be consistent with congestive heart failure (CHF). In CHF, the inability of the weakened heart to provide a sufficient cardiac output results in elevated filling pressures and subsequent pulmonary congestion and peripheral edema. The pleural effusion was drained and was found to be exudative, which suggested another underlying etiology, beyond CHF. The patient treated with a diuretic and an ACE inhibitor. A transesophageal echocardiogram (not shown) revealed hypertrophic cardiomyopathy, which is characterized by myocardial hypertrophy due to long-standing hypertension. Clinical indications of hypertrophic cardiomyopathy include dyspnea, angina and a bisfiriens carotid pulse. Five days later the chest X-ray shows the recurrence of the pleural effusion as well as significant cardiomegaly. The TEE shows right atrial inversion and a spot diagnosis of cardiac tamponade that correlates with the clinical findings. The MRI shows pericardial thickening and hemorrhage around the heart and the cytology in both the pleural and pericardial fluid was subsequently found to be positive for adenocarcinoma cells. A primary lung neoplasm is believed to have metastasized to the pericardium resulting in the cardiac tamponade.

Case 8

Diagnosis: Craniopharyngioma

Craniopharyngiomas are benign, slow growing tumours that commonly arise from the supra- or intrasellar region. Arising as remnants from Rathke's pouch, these tumours account for 3 to 5% of all intracranial neoplasms. Mechanical compression of the optic nerve results in visual field defects and an increase in intracranial pressure resulting in headaches. Both the anterior and/or posterior pituitary gland can be affected, commonly resulting in clinical signs of hypopituitarism. This patient presented with signs of diabetes insipidus due to loss of arginine vasopressin. Figure 8.2 is a coronal T1-weighted MRI with contrast, and clearly shows the neoplasm.

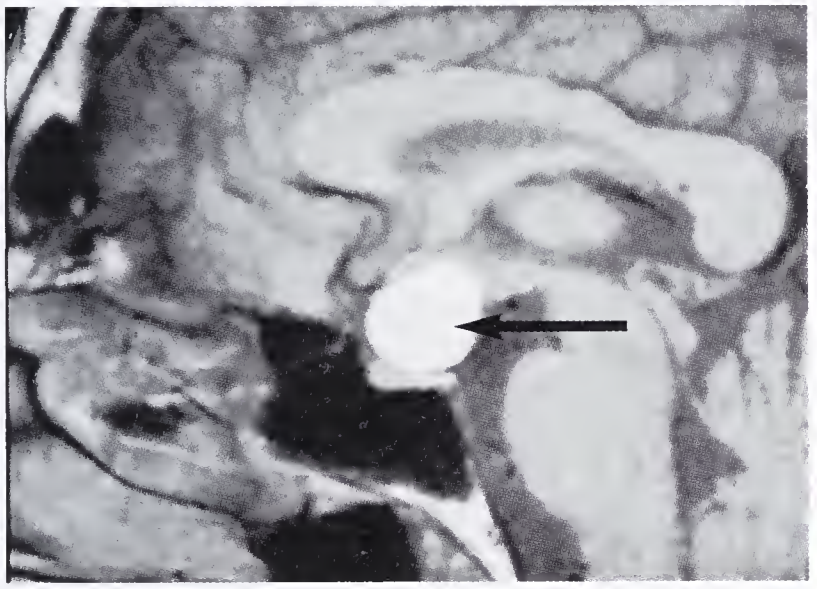

FIGURE 8.2. *MRI of pituitary gland with contrast.*

Case 9

Diagnosis: Primary Sclerosing Cholangitis (PSC)

Progressive inflammation and sclerosis of the extra- and intrahepatic biliary ducts characterize primary or idiopathic sclerosing cholangitis. It is commonly associated with inflammatory bowel disorders such as ulcerative colitis and Crohn's disease. Figure 9 shows the characteristic thickening of the common bile duct with a narrowed lumen, giving the appearance of 'beads on a string'. Common clinical presentations include jaundice, pruritus, and RUQ abdominal pain.

Case 10

Diagnosis: Hemolytic Uremic Syndrome

Her blood film is shown in Figure 10. Stool culture grew *E. coli* 0157:H7 and the diagnosis of HUS/TTP was made. The patient was transferred to a downtown hospital for plasmaphoresis. The patient's renal function improved over the next 5 days as seen by an increase in urine output and decrease in serum creatinine. Her platelets and hemoglobin returned to normal and there was a normalization of LDH.

Hemolytic uremic syndrome (HUS), along with thrombotic thrombocytopenic purpura (TTP), are disorders of thrombotic microangiopathy. HUS is a rare but serious complication of infection by Shiga-like cytotoxin-producing strains of *E. coli*. The diagnosis can be made by identification of the organism in the stool. It is characterized by acute renal failure, microangiopathic hemolytic anemia due to fibrin thrombotic occlusions in the glomerular capillary loops and systemic arterioles, as well as thrombocytopenia and anemia, caused by a deficiency of erythropoietin production in the failing kidney. It is often preceded by a prodrome of bloody diarrhea and abdominal pain. TTP is more often associated with fever and neurological manifestations. Evidence suggests that plasma exchange along with fresh plasma and corticosteroids may reduce remissions. The prognosis for recovery of renal function is generally poor.

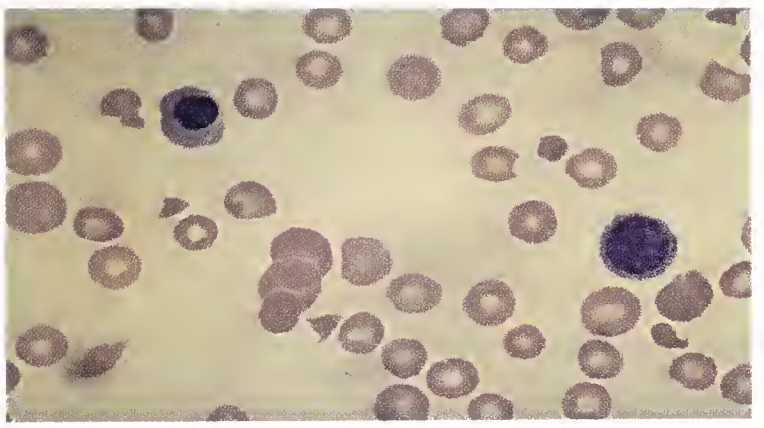

FIGURE 10. *Blood film showing lysed red cells and few platelets*

Inflammatory Bowel Disease

Chad G. Ball, M.Sc. (OT2), Alison Cooke, B.Sc. (OT2) and MaryAnn Cooper, M.D., F.R.C.P.(C)

Introducing Morning Report

Morning Report is a traditional round in most internal medicine training programmes. While the specific format of Morning Report varies between institutions, it typically involves the presentation of a clinical case followed by a case-oriented discussion. At the University of Toronto, the goals of Morning Report are to facilitate teaching and learning at various levels of medical training, to promote social interaction between the medical faculty and the medical housestaff, and to discuss issues related to local hospital structure and function. Traditionally, the medical team that was on-call the night prior to Morning Report presents a clinical case from the previous day's admissions. A staff physician then leads a discussion of the case with the goal of emphasizing the clinical reasoning process involved in the diagnosis and management of the patient's condition. Discussion focuses on the generation of a provisional and differential diagnosis for the presenting complaint, refinement of the provisional diagnosis as additional information becomes available, the anticipation of laboratory and radiological findings, and principles of management of the condition.

The Scene

A conference room in a Toronto teaching hospital. Medical students, residents and staff physicians are seated around a large conference table. Susy, a third year medical student, Bob, a fourth year medical student, Rhonda, a PGY-3 surgical resident and Terry, a PGY-4 medicine resident have selected an interesting case which Bob will present to the group. Dr. Jones, a staff physician, will facilitate the discussion of the case.

Bob: Mr. K. is a 33-year-old man who presented to the emergency department with a chief complaint of moderate abdominal pain, fever and extensive bloody diarrhea of 2 days duration.

Dr. J.: Let's pause here for a moment. Clinicians usually begin to formulate a differential diagnosis after hearing the patient's chief complaint. Susy, what is on your differential list when you hear the chief complaint of rectal bleeding with diarrhea?

Susy: The differential diagnosis of lower gastrointestinal tract bleeding can be approached by using major categories of disease such as carcinoma, structural abnormalities and various inflammatory conditions. While the majority of colon cancer is adenocarcinoma, colonic polyps are also usually included in this category as it is believed that tubular adenomas may act as pre-malignant growth sites. Structural diseases include anal fissures, internal hemorrhoids and various arteriovenous malformations such as vascular ectasias. Finally, inflammatory diseases of the colon include diverticular disease, infectious colitis, ischemic colitis, radiation colitis and of course, inflammatory bowel diseases such as ulcerative colitis (UC) and Crohn's disease.

Dr. J.: That is a very well organized and thorough differential diagnosis Susy! To help us focus our search, what other patient information would be helpful here Bob?

Bob: The patient should be asked to elaborate regarding his stool frequency, amount of rectal bleeding, abdominal cramps, abdominal pain, fecal urgency and tenesmus.

Dr. J.: I noticed that Bob made a distinction between abdominal cramps and abdominal pain. Why is that, Rhonda?

Rhonda: Well, "pain" is a broad term, whereas the term "cramps" typically refers to colicky pain with a remitting-relapsing pattern. It is possible, for example, to have significant intermittent abdominal cramping with no concurrent abdominal tenderness.

Dr. J.: Very Good! So Bob, can you provide us with more information about the patient?

Bob: The patient had been feeling well and had noticed nothing unusual except for a 5 pound weight loss in the past 2-3 weeks. Two days prior to his admission, he began to pass significant amounts of bright red blood per rectum. While Mr. K. had trouble quantifying it, he noted that the toilet water would be completely red after he had finished. This was accompanied by very loose stools and a strong sense of urgency and tenesmus. At this point he reported 5-6 bowel movements per day. He also noted moderate and diffuse abdominal tenderness and a left lower quadrant cramping relieved by defecation. Furthermore, upon taking his temperature, he observed a mild fever of 38.3°C. These symptoms remained consistent until his admission 2 days later.

Dr. J.: What does the timeframe of this patient's problem suggest to you, Susy?

Susy: Because Mr. K. had noted a significant weight loss over a relatively long period of time prior to his current complaint, I believe that there is most likely some underlying medical illness. I would tend to see this episode as an acute exacerbation of an undiagnosed chronic condition.

Dr. J.: Bob, what is the patient's past medical history?

Bob: Mr. K. has been relatively healthy with no history of similar problems. He has had no major medical illnesses with the exception of recurrent *Streptococcal* pharyngitis. He has had 2 surgeries on his left knee: one 5 years ago to remove damaged cartilage and the other 7 years ago to repair a torn ACL. While he has no cardiac risk factors, he does have a positive family history for inflammatory bowel disease; his paternal uncle was diagnosed with Crohn's disease at age 27.

Dr. J.: That brings up an interesting point. Is there a significant part of the history that we are missing which might help us with respect to ruling out inflammatory bowel disease, Terry?

Terry: A history investigating the extraintestinal manifestations is crucial to any discussion of inflammatory bowel disease (IBD). This includes comment on nutritional concerns such as weight loss, hematologic problems, in particular anemia, rashes and other skin manifestations, muscoloskeletal findings of arthritis, osteoporosis, or osteomalacia, renal abnormalities including kidney stones, and a host of other findings including fever, iritis, hepatic or biliary disease, and thrombophlebitis.

Dr. J.: Excellent. Bob, you alluded to weight loss and a mild fever in Mr. K. Does he have a history of any of these other abnormalities?

Bob: Yes, he does have some mild arthritis in his left knee. Furthermore, the general laboratory findings indicate anemia (hematocrit = 34%), increased sedimentation rate (ESR = 28) and reduced serum albumin (3.1 mg/dl).

Dr. J.: What's the next step with our patient, Bob?

Bob: I would do a thorough physical exam. Mr. K's physical exam was remarkable in presenting mild general abdominal tenderness on deep palpation. On rectal examination, bright red blood was evident, but no masses, fissures, or hemorrhoids were noted.

Dr. J.: Very good, Bob. It sounds like our suspicion of distal mechanical problems or colon cancer might be somewhat reduced. How could we attack the issue of colitis in our differential, Susy?

Susy: To rule out infectious colitis a stool specimen culture would be required. Specifically, we would look for *Salmonella, Shigella* and *Campylobacter*. We may also screen for ova and parasites as well as perform a stool toxin assay for *C. difficile*. We must be wary of gonorrhea, chlamydia, herpes and syphilis infections in sexually active patients with proctitis. Ischemic colitis is possible, but unlikely unless the patient is elderly with a history of cardiovascular disease. Finally, a negative history of radiation exposure to the abdomen and pelvis would rule out radiation colitis.

Dr. J.: And what about investigating the possibility of IBD, Rhonda?

Rhonda: Certainly the clinical picture and patient history are extremely valuable in distinguishing between Crohn's disease and ulcerative colitis. Crohn's disease, for example, is typically insidious in onset compared to ulcerative colitis. Furthermore, it is consistent with intermittent bouts of low grade fever, diarrhea, tenderness and masses due to ileal involvement, and right lower quadrant pain. Crohn's is also associated with much less rectal bleeding than ulcerative colitis, and sometimes none at all, but is consistent with a greater feeling of general malaise. Ulcerative colitis commonly displays anemia and low albumin concentrations due to blood loss, as well as lower abdominal cramps and urgency due to rectal inflammation.

Dr. J.: Is there any definite way to determine the presence of IBD, or more specifically to differentiate between Crohn's and ulcerative colitis, Terry?

Terry: The key to diagnosing IBD is a combination of direct and radiographic visualization. When suspicious of ulcerative colitis, a sigmoidoscopy is extremely useful as UC lesions begin at the anal verge of the distal rectum and proceed proximally to varying degrees. It is also ideal to begin with a sigmoidoscope in situations such as this because there is always a risk of perfora-

tion in severe ulcerative colitis. When suspicious of Crohn's disease, however, an upper-GI series with small bowel follow-through and then a colonoscopy is preferable. This is particularly helpful in identifying the strictures, fistulas and ulcerations common along all parts of the GI tract in Crohn's disease. A barium enema is also possible, but would not offer the advantage of obtaining a biopsy from the colon or terminal ileum.

To summarize, endoscopic findings for ulcerative colitis include rectal involvement, friable mucosa, continuous and diffuse involvement, edema and erosions. Crohn's disease would be evident as the presence of discontinuous involvement at multiple sites (usually the terminal ileum is affected), cobblestoning, linear ulcers and areas of stenosis (see Table 1).

Table 1
Comparison between Ulcerative Colitis and Crohn's Disease

Feature	Ulcerative Colitis	Crohn's Disease
Pathologic		
Discontinuous involvement	0	++
Transmural inflammation	0/+	+++
Deep fissures and fistulas	0	++
Confluent linear ulcers	0	++
Crypt abscesses	+++	+
Focal granulomas	0	++
Clinical		
Rectal bleeding	+++	+
Malaise, fever	+	+++
Abdominal pain	+	+++
Abdominal mass	0	++
Fistulas	0	+++
Endoscopic		
Diffuse, continuous involvement	+++	0/+
Friable mucosa	+++	0/+
Rectal involvement	+++	+
Cobblestoning	0	+++
Linear ulcers	0	++

Dr. J.: What were Mr. K.'s findings on these exams, Bob?

Bob: A sigmoidoscope was performed and an edematous, friable terminal ileum and rectum were noted. Mucopus was also evident. Upon colonoscopy, no proximal involvement was observed and a biopsy sample was taken from the sigmoid colon. Finally, X-rays of the colon showed multiple irregular serrations in the transverse colon representing mucosal ulcerations and the presence of pseudopolyps. A barium enema was not performed due to its limited contribution in ulcerative colitis patients, and due to the risk of precipitating toxic megacolon in patients with severe disease (see Figure 1).

Figure 1. *X-ray of toxic megacolon shows massive dilatation of the colon secondary to complete cessation of colonic neuromuscular activity.*

Dr. J.: Taking into account the history of our patient as well as his physical findings and lab tests, what do you think, Rhonda?

Rhonda: I believe this clinical picture makes a very strong case for a diagnosis of ulcerative colitis.

Dr. J.: What would you expect to see on the pathology report for the colonic biopsy Terry?

Terry: Significant mucosal/submucosal inflammation with multiple crypt abscesses is consistent with ulcerative colitis. For Crohn's disease, however, I would have expected evidence of transmural inflammation with deep fissures and fistulas, confluent linear ulcers, focal granulomas and creeping fat (see Figure 2).

Bob: The pathology report for Mr. K. is consistent with ulcerative colitis.

Dr. J.: That was a very good discussion of IBD and its manifestations! Very briefly Rhonda, can you tell me how you would approach Mr.K's treatment?

Rhonda: There are two main objectives in the treatment of IBD: First, to terminate his acute, symptomatic attack

and second, to prevent future attacks. The treatment of acute ulcerative colitis depends upon the extent of colonic involvement and the severity of the illness. It is important to keep in mind that ulcerative colitis, unlike Crohn's, is completely curable with a colectomy, but most patients consider this a last resort. While no single drug is curative, ASA, antibacterial compounds, corticosteroids, antispasmadics and sedatives in varying combinations are helpful. In severe cases, immunosuppressants are indicated.

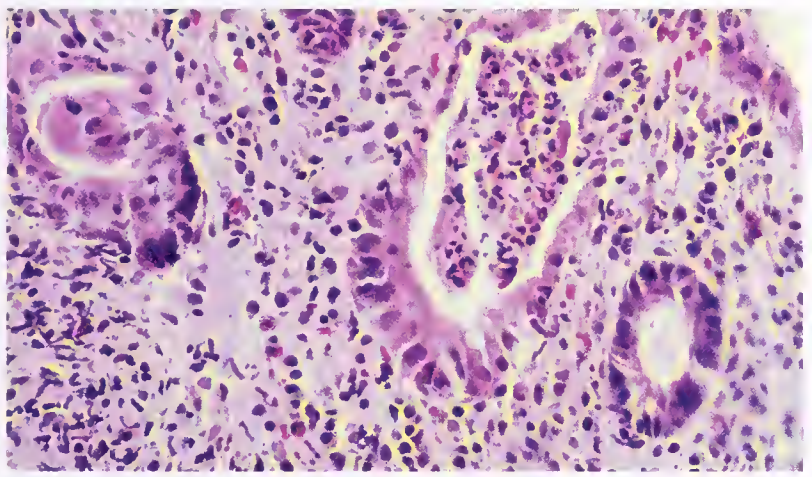

Figure 2. *Ulcerative colitis. 1. Active colitis with cryptiti and crypt abscesses. 2. Crypt architecture distortion. 3. Mucin depletion (absent goblet cells). 4. Lymphoplasmacytic infiltration of lamina propria along with eosinophils and polys.*

Dr. J.: Very good approach. Susy, can you tell me if there is an increased risk of colon cancer in these patients that deserves our vigilance?

Susy: Yes there is. In ulcerative colitis patients with disease proximal to the sigmoid colon, there is a dramatic increase in risk of developing colon cancer. In patients with a colitis history of 10 years or longer, the risk increases approximately 0.5-1 % per year. For screening purposes, these patients should have colonoscopies every 1 to 2 years, beginning 8 to 10 years after diagnosis. Also, increased ingestion of folic acid is recommended due to its association with a decreased risk of cancer development.

Dr. J.: And what about the prognosis of our patient in a general sense Terry?

Terry: Although ulcerative colitis is a lifelong disease characterized by exacerbations and remissions, it can typically be well controlled with medical management, without the need for surgery. As a result, most ulcerative colitis patients, with the proper support, can lead productive and near normal lives.

Dr. J.: Fantastic group! Thank-you for presenting such an interesting case and engaging in a very thorough discussion.

Acknowledgements

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Clinical Pearls

1. Patients with IBD can present with very nonspecific signs and symptoms. A comprehensive history and physical examination are important in directing appropriate investigations.

2. Emergency surgical management of IBD is indicated in cases of gross hemorrhage obstruction, perforation and/or toxic megacolon.

3. IBD is a lifelong disease characterised by exacerbations and remissions. As such it may have profound emotional and social impact on the patient. IBD-specific social support should be encouraged.

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ROSS MEMORIAL HOSPITAL

The Health Services Restructuring Commission has directed that Ross Memorial Hospital will play a strengthened role in the delivery of health care in Victoria County and the surrounding area, promoting the expansion and enhancement of services.

Positions are available immediately in Family Medicine and Emergency Medicine, and opportunities exist in some specialty areas. The hospital has 155 beds to be increased to 218 by 2003.

A state-of-the-art birthing unit was recently completed. Ross Memorial Hospital in Lindsay, Ontario serves a population of approx. 70,000, and lies in the heart of the Kawarthas, offering an outstanding lifestyle and a wide range of urban amenities.

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PET and fMRI: Clinical Tools of the Future?

Chris Heyn, BSc. (MD/PhD2) and Michael C.Y. Chang, MSc. (0T2)

Abstract

The understanding of the functional anatomy and physiology of the human brain has undergone tremendous advancement in recent years. One of the factors contributing to this explosion of knowledge has been the development of sophisticated non-invasive imaging techniques, particularly Positron Emission Tomography (PET) and Functional Magnetic Resonance Imaging (fMRI). Despite the advancements made in these areas, and the wealth of knowledge that has come with them, there has been little use of these technologies in the diagnosis and treatment of human diseases. This article briefly reviews the science of PET and fMRI, and examines the potential role that these technologies may play in the fields of neurosurgery, psychiatry, and neurodegenerative disease and stroke. The article concludes with a brief look at the future of functional imaging as a clinical tool with a discussion of the barriers that still exist in their implementation.

Introduction: Technical Basis of Pet and FMRI

PET uses systemically administered radiotracers labeled with short-lived positron emitting isotopes to track chemical transformation in living systems. For instance, the radiotracers 18F-fluorodeoxyglucose and 15O-water can be used to measure regional cerebral glucose metabolism and regional cerebral blood flow, respectively. One of the major advantages of PET is the ability to measure radioisotope concentration in the nanomolar-picomolar range. Another advantage of PET is its biochemical selectivity in that specific radiotracers can be designed to bind selectively to molecular targets, such as receptors, transporters, or enzymes involved in the synthesis or metabolism of neurotransmitters. Not only has PET been used for biochemical and pharmacological imaging of the human brain, but it can also be used to assess cerebral function through measurements of regional glucose utilization enabling the investigation of cognitive processes.

Unlike PET, fMRI can be performed without the injection of exogenous agents. The basis of fMRI is the detection of changes in proton relaxation derived from magnetic field heterogeneities within the tissue. MRI systems are designed to generate as uniform a magnetic field as is physically possible. Heterogeneity in the magnetic field is undesirable and results in a decrease in the signal generated from tissue being imaged. There are circumstances where the magnetic field homogeneity is greatly disturbed. This usually happens when a tissue has a large concentration of iron-containing compounds, such as hemoglobin. In particular, deoxyhemoglobin contained in red blood cells is quite adept at increasing the magnetic heterogeneity of a tissue. During neuronal activation, there is an increase in regional cerebral blood flow, regional cerebral blood volume, and tissue oxygenation. These changes lead to a local decrease in the concentration of deoxyhemoglobin and a decrease in the magnetic heterogeneity that increases the local MR signal. By comparing MRI images during rest with those obtained during the execution of a task, statistically significant changes in MR signal can be mapped and correlated with brain activation. This form of fMRI is also known as blood oxygen level dependent (BOLD) imaging and is the most prevalent form of fMRI used today.

Application in Neurosurgery

The application of functional imaging to neurosurgery has shown promise in a number of areas. Firstly, functional imaging can be used to plan surgery by localizing functionally eloquent cortex. Currently, direct electrocortical stimulation is the gold standard for identification of eloquent cortex. This is done to minimize postoperative morbidity and improve outcome by helping to define a surgical plan that maximizes resection of the lesion while minimizing the risk of neurological sequelae. Numerous studies have demonstrated the utility of fMRI and PET in this area. In some respects, fMRI and PET are superi-

or to direct electrocortical stimulation since the entire brain can be studied, and in cases where patients are not appropriate for awake craniotomy thus precluding the use of direct stimulation. In the case of language localization, direct stimulation is inadequate because both hemispheres cannot be evaluated for hemispheric dominance. In fact fMRI is as reliable as the Wada test for determining language dominance.

Functional imaging can also provide preoperative information about brain tumors that can influence treatment strategy. Some neurosurgeons, for example, state that intra-axial tumors may be resected with a low risk of neurological deficit if the tumor removal stays within the confines of the grossly abnormal tissue. Recently, this point of view has been contested by studies that have shown some tumors to contain functional cortex. In addition, other abnormal tissues, such as arteriovenous malformations, have also been shown to contain functional cortex (see Figure 1). Furthermore, PET studies have been used to distinguish actively growing tumors. PET and fMRI could therefore theoretically provide useful information to the neurosurgeon prior to resection or correction of abnormal tissue.

Figure 1. *fMRI image showing activation (in white) of the primary motor cortex in an arteriovenous malformation (AVM) in the right parietal cortex. The task was left finger tapping.*

Application in Psychiatry

In the area of psychiatry, applications of PET outnumber those for fMRI owing mainly to the fact that PET has very high biochemical sensitivity and selectivity, whereas fMRI does not. In the study of depression, for instance, PET has been used to map brain areas associated with depressive illness or to define the neurochemical correlates of the illness and its treatment. Regional deficits of functional activity have been consistently detected in the brains of subjects with ongoing affective symptoms, particularly in prefrontal areas. The development of relevant radiotracers, particularly for select serotonin and dopamine receptor subtypes, for use with PET has improved our understanding of the neurochemistry of mood disorders. Furthermore, PET has also provided researchers with tools to study a variety of mental states, including hallucination, and also the possibility that defective interactions among distributed brain areas may underlie certain dysfunctions of conscious integration, such as those seen in schizophrenia. In addition, fMRI has made headway in the study of schizophrenia with a recent study that provided convincing results on the location from which auditory hallucinations originate in patients with chronic schizophrenia.

Application in Neurodegenerative Disease and Stroke In the field of neurodegenerative disease, PET and fMRI have shown some clinical promise. In Huntington's disease, for instance, PET can measure striatal D1 and D2 dopamine binding in order to monitor rate of loss of striatal function in preclinical and clinical disease, to monitor treatment intervention, and to identify asymptomatic Huntington's disease mutation carriers who are actively progressing and who would thus be suitable for putative neuroprotective therapies. PET can also offer the opportunity to improve a diagnosis of dementia by providing regional functional measurements, which can be used to substantiate clinical judgment. In Alzheimer's disease, PET has demonstrated progressive reductions in resting-state brain glucose metabolism and blood flow in relation to dementia severity, more so in association than primary cortical regions. Moreover, in the area of stroke, both PET and fMRI have been used to monitor the restoration of cortical plasticity or adaptation. Recently, the utility of fMRI in monitoring cerebral activation of motor function in post-stroke hemiparesis has been evaluated in a pilot study. It is conceivable that in the future, functional imaging may be used to guide rehabilitation efforts in this disease.

Non-Nuerological Applications

Functional imaging, particularly PET, has also been applied to other illnesses unrelated to the brain. For instance, PET may represent a new and noninvasive diagnostic procedure for the detection and the staging of pancreatic cancer. PET could also help predict the chances of relapse of a residual mass of lymphoma after completion of chemotherapy. In addition, PET allows the non-invasive functional assessment of myocardial perfusion, substrate metabolism and cardiac innervation, as well as the study of skeletal muscle metabolism and perfusion in vivo. In the detection of myocardial viability, PET is regarded as the gold standard, and it is the only method available for the quantitative assessment of myocardial blood flow.

Future Perspectives

This article has thus far highlighted the success of fMRI and PET in a number of studies that have demonstrated their clinical utility. Despite this success, however, the question still remains as to why we have not seen their implementation into the clinical setting. In all fairness, fMRI has only been in use for a short while, having only been developed in the last decade or so. On the other hand, PET has been around for almost fifty years and has still not found widespread use in clinical practice. Practical and technological considerations may in part be responsible for the underutilization of functional imaging in clinic. In the case of neurosurgical applications of functional

Presently, a number of limitations exist. Currently, most neurosurgical procedures are quite invasive and performed with craniotomy. There is no real advantage to using functional imaging when direct cortical stimulation is sufficiently effective. In addition, during craniotomy, bone and tumor removal cause large brain shifts to occur rendering preoperative data unreliable and necessitating direct stimulation. Recent studies have also demonstrated that certain lesions may produce erroneous fMRI data by producing pressure effects or changes in vascular autoregulation. Eventually, however, as neurosurgery becomes less and less invasive through the use of catheter-like appliances under real-time MRI guidance, direct cortical stimulation may not be feasible. It is conceivable that at this time, fMRI and PET will replace cortical stimulation in assisting neurosurgeons in defining eloquent cortex, making this technology a necessity.

With respect to PET, there are other broader technical limitations to consider. Unlike fMRI, which uses single subjects, PET requires the analysis of a group of subjects in order to arrive at a conclusion about a pattern of activation. Obviously, this technical limitation severely limits the practicality of using PET in clinical diagnosis. Nevertheless, single-subject 3-D PET techniques are being developed that appear to be comparable to fMRI. PET also requires the administration of intravenous radioactive tracers – a feature that makes this imaging modality less appealing as a diagnostic procedure.

Cost and availability issues may also be factors limiting the use of functional imaging. This is especially the case with PET, where special hardware and facilities are required. fMRI, on the other hand, can be performed on standard 1.5-tesla clinical MR scanners. However, availability still remains an issue in Canada, a country with the lowest number of MRI scanners per capita of any of the developed nations.

Finally, before functional imaging ever becomes part of routine clinical practice, a paradigm shift in the way clinicians view the radiological diagnosis of disease has to occur. Traditionally radiology has been limited to correlating static structural pathology with disease and has virtually ignored the dynamic aspects. In many diseases that involve the brain, such as Alzheimer's and many of the psychiatric illnesses, structural alterations alone may not be sufficient enough for diagnoses. In these cases, functional data may prove to be a valuable addition to the diagnostic repertoire. Of course, better knowledge of functional changes in these diseases is needed. Therefore, the better we understand the molecular mechanism leading to pathology in the brain and other organ systems, the better we will be able to physically visualize a management plan with the use of PET and fMRI.

Acknowledgements

The authors would like to thank Dr. David Mikulis for providing the fMRI image and for his help and comments during the preparation of this manuscript.

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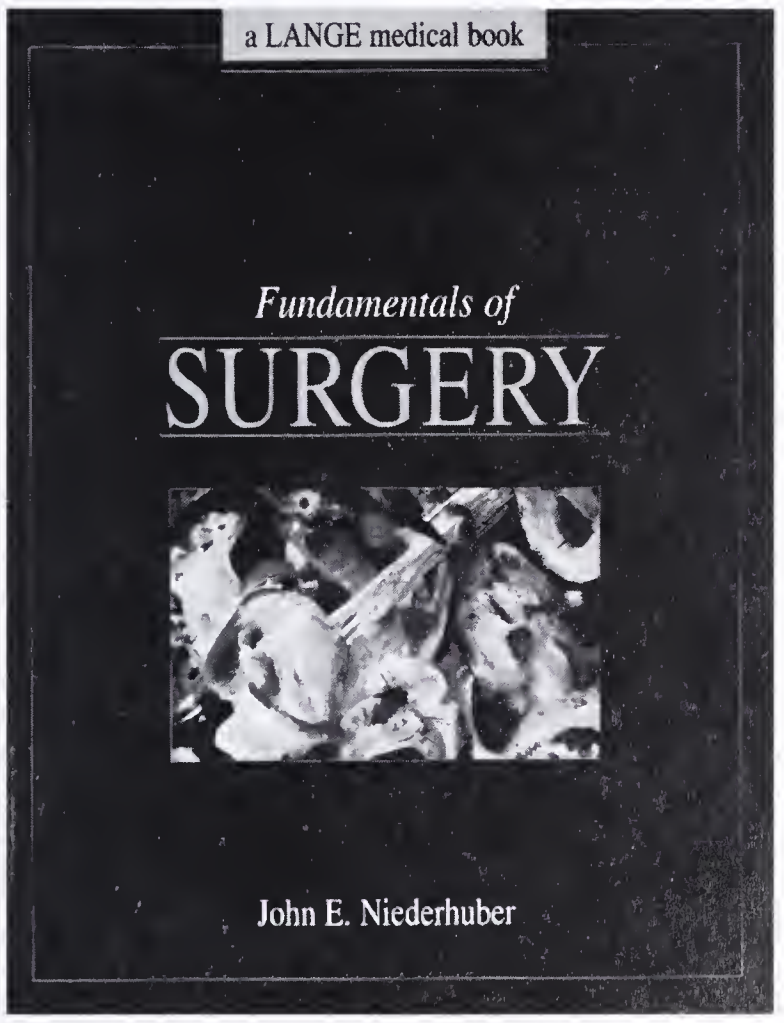
UTMJ Reviews – Surgery

Fundamentals of Surgery

Reviewed by Tara Abrahamsohn, B.Sc. (0T1)

Edited by: John E. Niederhuber
Publisher: Appleton & Lange
Date of Publication: 1998
Number of Pages: 836
Cost: Paperback Edition, $69.70 (CDN)

Strengths:	Excellent introduction to the world of surgery; prepares the reader for the expectations of a surgical rotation and how best to survive
Weaknesses:	Summaries of pathogenesis and etiology of disease are brief and occasionally require prior knowledge of the disease
Target audience:	Undergraduate medical students (primarily clerkship years)



"Surgery is the oldest method of dealing with human disease. It originated not as a science but as an art, long before there existed an understanding of pathology, physiology and the principles of diagnosis. Today it is 'the art' of surgery that sets it apart from the other fields of medicine."

– John E. Niederhuber

For many medical students entering clerkship, the beginning of surgery is filled with apprehension and uncertainty. *Fundamentals of Surgery* attempts to alleviate this apprehension by providing a broad base of information regarding surgical diseases and assisting the student in making the transition from the classroom to clerkship. The book, in its first edition, is one of many in the series of LANGE medical books. Chapters are authored by American surgeons specialized in a given surgical field. To make the book unique among surgical reference books, historical facts have been interspersed throughout. Each chapter begins with a half page box filled with Key Facts, which unfortunately are too basic and brief to provide a concise summary of the chapter.

The first third of this book is an excellent introduction to surgery for any clerkship student. It gives the student an idea of what to expect on the ward and in the operating room. The book provides information regarding admission histories and physicals, operating room notes and post-operative notes. There is also an extensive chapter detailing a system-by-system approach to the Medical Assessment of the Pre-operative patient. The beginning of the book also discusses basic physiology such as Acid-Base Metabolism and Fluid compartments. Overall these chapters provide a great review prior to any surgery rotation.

The remainder of the book focuses on surgical presentations. Each chapter discusses a particular organ, organ system or specific surgical subspecialty. These chapters are well organized and all begin with a basic review of anatomy and physiology. The authors then discuss the diagnosis and management of specific diseases. Little emphasis is placed on pathophysiology and etiology of disease. For the student considering a career in surgery, there is little information on specific procedures. Each chapter focuses on important diseases, both non-neoplastic and neoplastic, and tends to avoid the obscure.

A wide range of topics are covered including Orthopaedics, Neurosurgery, Plastics, Urology, Surgical Oncology, Paediatric Surgery and Transplantation. In fact there is quite an extensive section on Transplantation including chapters on Kidney, Liver, Cardiac and Lung Transplants. This is in excess of what is required at the clerkship level.

Overall *Fundamentals of Surgery* is a good reference for the clerk not interested in a career in surgery. It is easy to read, provides diagnosis and management algorithms and presents information at a level that is understandable and appropriate for clerkship. *Fundamentals of Surgery* has many components of a good text: it is an excellent introduction to surgery, with clear and concise management plans. However, it falls short in discussing the pathogenesis and etiology of disease. *Fundamentals of Surgery* is thus comparable but not superior to other introductory surgical texts. For the student interested in pursuing a career in surgery, this book is a great starting block, but this student will require a second book detailing surgical procedures.

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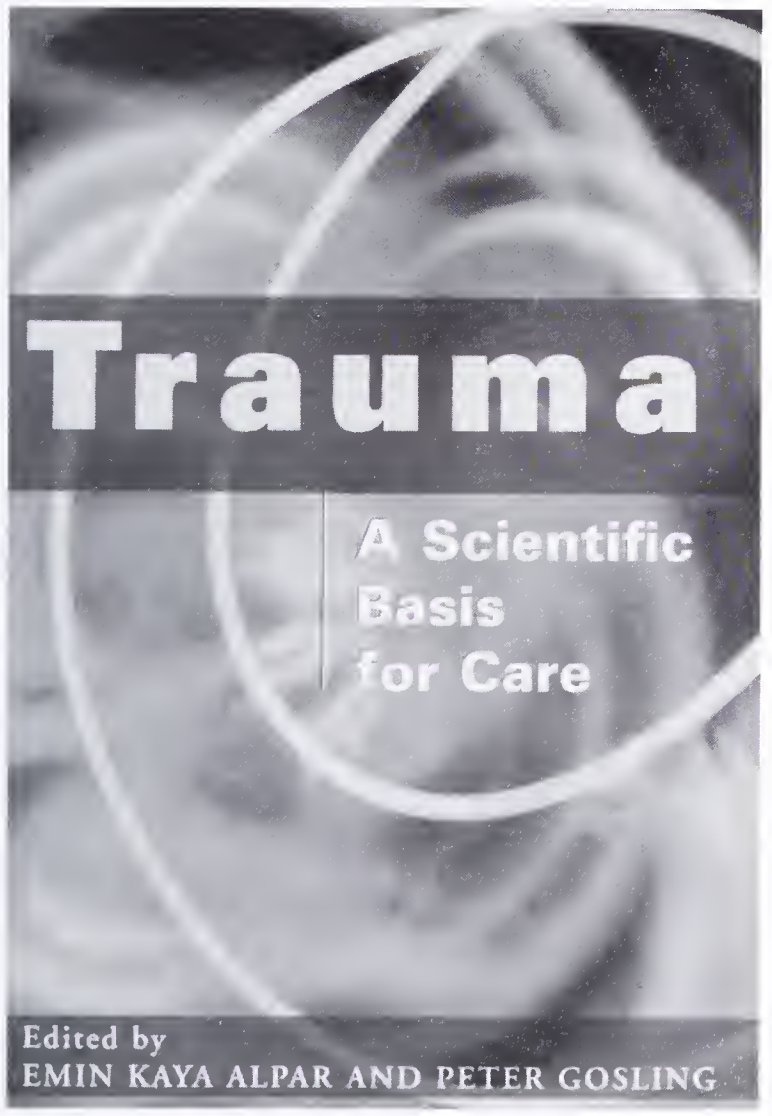
UTMJ Reviews – Surgery

Trauma: A Scientific Basis for Care

Reviewed by Gordon Buduhan, B.Sc. (0T0)

Edited by: Edited by Emin Kaya Alpar and Peter Gosling
Publisher: Arnold.
Date of Publication: 1997
Number of Pages: 383
Cost: Hardcover edition, $157.95 (CDN)

Strengths:	Wide scope of topics relevant to trauma with emphasis on pathophysiology
Weaknesses:	Organization of chapters is inconvenient
Target audience:	Residents, fellows, staff physicians with interests in trauma and critical care



Trauma care, like every other area of medicine, continues to evolve and change. Recently, the specialty of trauma has borne witness to new developments that might radically change the current practice of clinical trauma management. While the familiar "ABCs" mantra continues to be the cornerstone of trauma care, novel hypotheses such as delayed fluid resuscitation and inflammatory modulation are currently being investigated through both laboratory and clinical studies in academic trauma centres worldwide. The importance of understanding the pathophysiologic processes at the cellular level and its subsequent implications in clinical practice is the driving force behind the writing of *Trauma: A Scientific Basis for Care.*

As stated in the Preface of the text, this book is not meant to be an exhaustive reference on trauma. Instead, the authors intended to highlight key areas of trauma care and their underlying physiologic and cellular mechanisms. Their product is a book that covers a comprehensive scope of topics: in less than 400 pages, virtually all of the major areas of trauma and critical care are discussed in varying degrees of detail. To achieve such brevity, the authors assume a more than rudimentary level of trauma and critical care aptitude of their audience. The obvious areas of strength of the book are the chapters on inflammation and the metabolic, circulatory and immune

responses to injury. The current understanding of the pathophysiology of these various mechanisms is exceptionally well written, with appropriate diagrams to further highlight key points. The authors provide a wide array of recent results from experimental and clinical studies, and the result is an extensive presentation of the current theories of traumatic and post-traumatic pathologic processes and therapy.

The organization of chapters is somewhat odd, as important subsets of a particular subject may be found in entirely different chapters. For example, the chapter entitled "Shock" is a mere 5 pages in length and gives only a brief generalization of the subject of delayed fluid resuscitation, a new and radically different concept in the management of haemorrhagic shock. With further searching, the landmark clinical and experimental findings are found in the chapter on pre-hospital care. The reader must refer to multiple chapters when studying a particular topic because many related concepts are dispersed in a sporadic fashion throughout the text. For example, "Pathogenesis of Infection" is Chapter 8, but "Sepsis in the Intensive Care Unit" is Chapter 17, and "Antibiotics and Antibiotic Resistance" is near the end of the book as Chapter 29. A practical suggestion would be to keep related chapters in closer proximity to each other, perhaps within a distinct encompassing section of the text such as "Infection and Sepsis."

Each chapter ends with a concise summary that efficiently highlights major points. The tables and diagrams included are effective in stressing key topics, and black and white photographs complement many of the chapters on neurosurgical and orthopaedic trauma. Again, each chapter is up-to-date with recent literature citations. The book includes a chapter on trauma epidemiology, using mostly statistics from the United Kingdom, but also presents some North American and international statistics for comparison. In keeping with the book's emphasis on scientific research, a chapter on biostatistics is included for review of basic principles of study design and analysis.

Overall, *Trauma: A Scientific Basis for Care* is an excellent trauma text that effectively highlights the current understanding of the pathophysiology of injury and demonstrates its clinical implications. It is relatively less expensive than many of the current trauma texts available and includes most of the relevant major subjects. However, students, residents and clinicians looking for a more extensive text on trauma may be disappointed with the book's focus on pathophysiology. Nonetheless, the book would serve as a superb supplementary reference for residents, fellows and staff physicians with a clinical or research interest in trauma and critical care.

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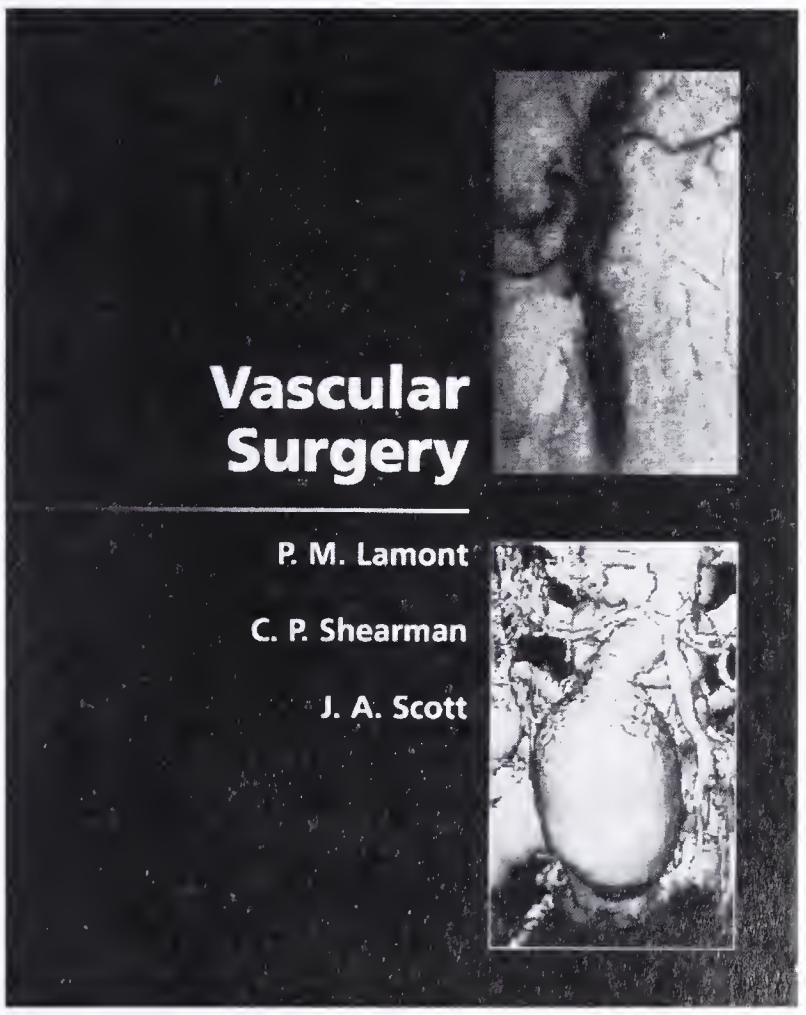
UTMJ Reviews – Surgery

Vascular Surgery

Reviewed by Martin Jugenburg, B.Sc. (0T1)

Written by: P.M. Lamont, C.P. Shearman and J.A. Scott
Publisher: Oxford University Press
Date of Publication: 1999
Number of Pages: 140
Cost: Hardcover edition, $201.95 (CDN)

Strengths:	Excellent overview and summary of the traditional knowledge and literature in vascular surgery; well organized and readable; illustrations and schematic drawings are very helpful
Weaknesses:	Not a comprehensive text; basic science relevant to vascular pathology is not covered well
Target audience:	Surgical trainees and surgery-oriented medical students



Great strides have been made in the field of vascular surgery. Many of these advances are due to the improvement in anesthesia, the development new antibiotic agents, a better knowledge of blood and fluid balance, a continually evolving concept of antisepsis, and the advent of anti-clotting drugs. *Vascular Surgery* by Lamont *et al.* is an up-to-date book that covers the basics of vascular surgery as well as recent advances in surgical technology in a concise way. It delivers 100 percent in each of these aspects, and therefore should be of interest to medical students and surgical trainees.

The book focuses on the principles, techniques, and results of vascular surgery procedures. There are fourteen chapters that cover all major aspects of vascular surgery, including an introductory chapter of 'Clinical assessment of the vascular patient.' At 140 pages and 53 plates, this is a very easy and fun to read book. This book is very well organized, and the text is accompanied with great photographs and illustrations.

After an opening chapter on history and physical examination relevant to the vasculopath, there are excellent chapters on investigative procedures and interventional

radiology. Later chapters are based on various clinical conditions, such as carotid artery stenosis, upper limb disease, lower limb arterial disease and vascular trauma, rather than specific surgical procedures. Pictures are used effectively to demonstrate investigations and show some of the typical pathologic findings, such as vessel occlusion on arteriography. Chapters covering specific clinical scenarios are well organized, and the relevant disease, injury, malformation and degenerative changes are described briefly. Appropriate investigations and management techniques which have proven most efficacious are described. As such, this book provides an initial approach to vascular problems and prepares the medical student or surgical trainee for the clinic or the OR. On the negative side, it does not give an overview of the recent research in areas such as cellular and molecular biology of the arterial wall, and circulatory physiology.

There are numerous texts on vascular surgery from which one can chose. On one end of the spectrum are the massive all-encompassing texts covering vascular surgery in general, or books dealing with more specialized topics such as atherosclerosis or amputation. On the other end of the spectrum are very brief, summary-note style review books meant for exam preparation, often used by students as quick references. *Vascular Surgery* fits between these two extremes, as it is a well written and easy to read book, yet it is brief enough that it can serve as a quick reference and a primer on major clinical issues one deals with in vascular surgery.

Overall, this text does provide an excellent foundation in vascular surgery, and is a very good introduction to the more comprehensive texts. I highly recommend this text to all trainees. The only problem with this book is an economic one: although this an exceptional book, the price is too steep, especially since it is intended for students.

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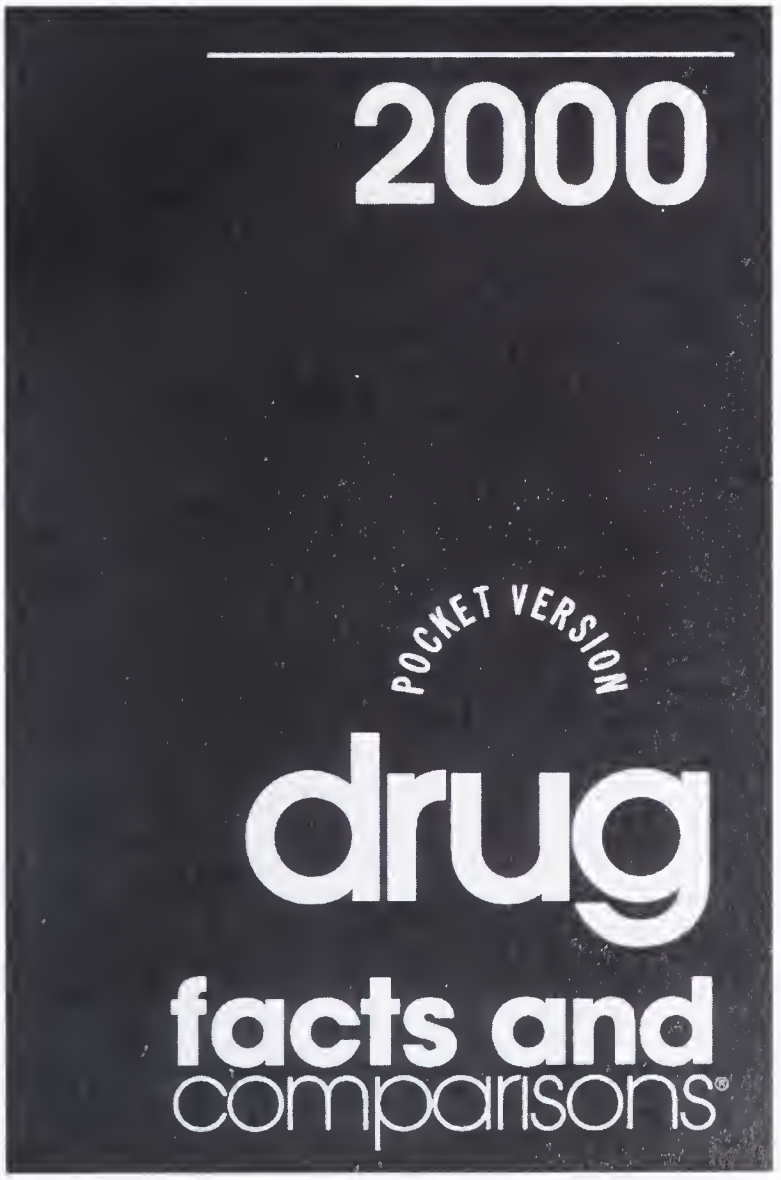
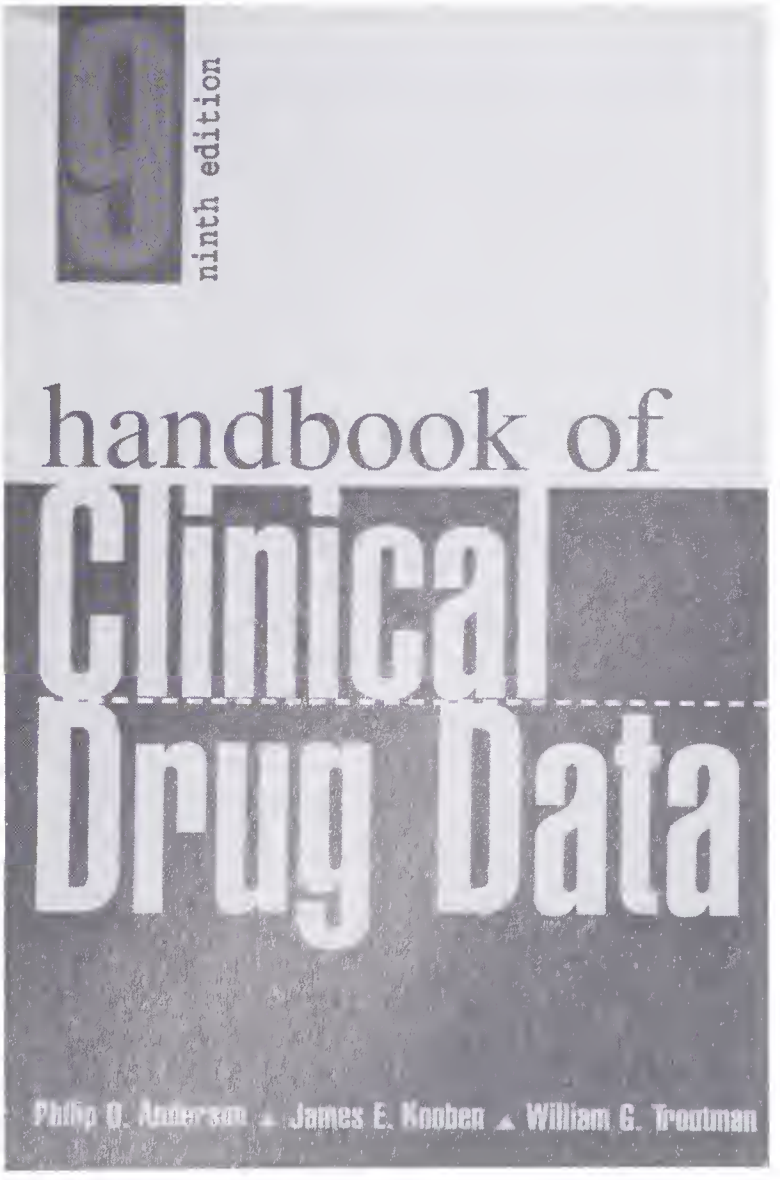
UTMJ Reviews – The Essentials

Reviewed by Amy S.M. Tam, B.Sc. (0T2) and Adrienne C. Vraets, B.Sc. (0T2)

Drug Facts and Comparisons Pocket Version
Written by: Facts and Comparisons Publishing Group
Publisher: Facts and Comparisons
Date of Publication: 2000
Number of pages: 1020
Cost: Paperback edition, $85.50 (CDN)

Handbook of Clinical Drug Data, 9th ed.
Written by: Philip O. Anderson, James E. Knoben and William G. Troutman
Publisher: Appleton & Lange
Date of Publication: 1999
Number of pages: 1104
Cost: Softcover edition, $69.70 (CDN)





clerks, residents and physicians alike must maintain a knowledge base of the growing number of available drug therapies. In this edition of the *UTMJ*, we examine whether pharmaeotherapy handbooks provide enough information to adequately arm students and clinicians for clinies and wards. Our criteria for review eneompassed both our essential and desired characteristies based on the reviewers' limited knowledge of elinieal pharmacology. The essential eriteria included: an easy fit in the pocket of lab eoat, tabs and a thorough index, generous use of tables, brevity yet adequate detail for patient eare and provision of referenees, statistics and evidenee-based information when possible. On our wish list were Canadian drug listings, drug costs, patient information, and data on over the counter drugs.

The *Drug Facts and Comparisons Pocket Version* is a condensed version of the original 4096 page *Drug Facts and Comparisons*.[1] It contains a wealth of information for its size, and is organized according to drug uses. There are ehapters on nutritionals, the major body systems, antibioties, topicals, antineoplastics and miseellaneous drugs. Within each chapter, drugs are organized aeeording to class. Turning to a specific class, readers initially find a table of generic names and the corresponding routes and doses available, trade names and pharmaceutical manufacturers. Next, serious warnings are included in boxes. Pharmaeology and pharmacokineties follow, with indications (labelled and unlabelled), and information on contraindications, speeifie disease states, pregnancy, laetation and paediatrics, precautions, drug interactions and adverse drug reaetions ineluded next. The reeommended indications, initial and maintenanee doses of eaeh drug are then listed for infants, ehildren and adults. The brief 14 page appendix is notable for its summary of formulae, management of overdoses and aeute hypersensitivity reaetions, and laboratory values.

Strengths of this referenee inelude its brevity and tables which instantly provide readers with important data, sueh as equivalent doses, onset and duration of aetion, half lives and the effeets of impaired renal excretion. The information is written elearly, and generally data are not difficult to find from the index, although the index eontains only generie drug names and U.S. trade names.

On closer examination, one finds some shorteomings that are sometimes eannot be forgiven. While the pages of the book are tabbed, a master key is not provided. Pharmaeology diseussions are restrieted to physiology, as in the original version,1 which may be disappointing to those whom enjoy having an understanding of bioehemistry to advanee their knowledge about potential drug interactions and side effects. The book would also be improved with more consistent notation and methods of presenting information. Tips like the inhibition of CYP3A4 by grapefruit juiee and the importanee of fish allergy with protamine sulfate were missing. An independent seetion on drug interaetions is much needed to reduce spaee and the work needed to cross referenee drugs. In an era of evidenee based medieine, data on mortality and morbidity reduetion are sorely missed, sinee these statisties often determine the optimal drug elass for an individual. Antidotes are dealt with poorly. Canadian drug names, indieated in the original version,1 are not included.

While the *Drug Facts and Comparisons Pocket Version* does provide much of the basie information needed to preseribe, it has design problems that make it more difficult to use than other drug handbooks. Its lack of referenеing and Canadian content, and its steep price may leave buyers thinking about what they are missing, rather than optimal therapies for their patients.

Appleton and Lange's *Handbook of Clinical Drug Data* is divided into 3 main seetions: Drug Monographs, Clinieal Information, and Appendiees.

The Drug Monographs are organized into eategories by drug uses. The eategories are tabbed for easy loeation. Within eaeh monograph, generie and eommon proprietary names are listed. There follows a deseription of: pharmacology, administration and adult dosage, speeial population dosages, dosage forms, patient instructions, pharmacokineties, adverse reactions, contraindieations, precautions, drug interaetions, parameters to monitor and other notes. When several drugs exist within a class, charts comparing dose, pharmaeokinetie, and side effeet parameters are usually given. Unfortunately, the eharts laek priee eomparisons. Overall, the Monographs seetion gives a high level of detail for a pocketbook, yet is elearly organized so the reader ean seleet and read only the paragraphs desired.

The Clinieal Information has subseetions eovering drug-indueed diseases, drug interactions and interferences, drug use in special populations, immunization, medieal emergeneies, nutrition support. The first three subseetions are partieularly good. They are extensive but readable, and often show data in ehart format. The Clinieal Information is truly a reference because it is not simply a eompilation from the Monographs. For example, the subsection on drug interaetions gives long lists of indue-

ers, inhibitors, and substrates of nine cytochrome P450 enzymes. The subsections on drug-induced diseases, drug interactions and interferences, and drug use in special populations have one major drawback: a lack of cross-referencing to the Drug Monographs. The Clinical Information on immunization and emergencies is not as useful as that in the preceding subsections. The vaccination information provided is American. Also, since emergency protocols are often particular to an institution, procedures listed in the book can likely only be used as a guide.

The Appendices give conversion factors, anthropometrics, pharmacokinetic equations, laboratory indices, and information on drug-laboratory test interferences.

There are some areas where enhancements could be made. More thorough coverage of over-the-counter preparations would be useful. Evidence and statistics on drug efficacy should be stated explicitly. (Although this information could be obtained from one of the hundreds of references appearing in each *Handbook* subsection.) Improvements might also be made in the area of Canadian content. For an American publication, the Handbook makes a fair effort to include Canadian drug names in the index. However, it gives no indications of drug availability in Canada or of Canadian recommended doses.

In summary, the *Handbook of Clinical Drug Data* is well-written and well-organized, with certain outstanding features (easy-to-navigate format, use of charts, presence of references). The shortcomings this book does are common to most competing publications. Altogether, the 9th edition of the *Handbook* is good. With improvement in some key areas, a 10th edition could be excellent.

References

1. *Drug Facts and Comparisons, 1999 edition.* St. Louis: Facts and Comparison, 1998. $174 (CDN).

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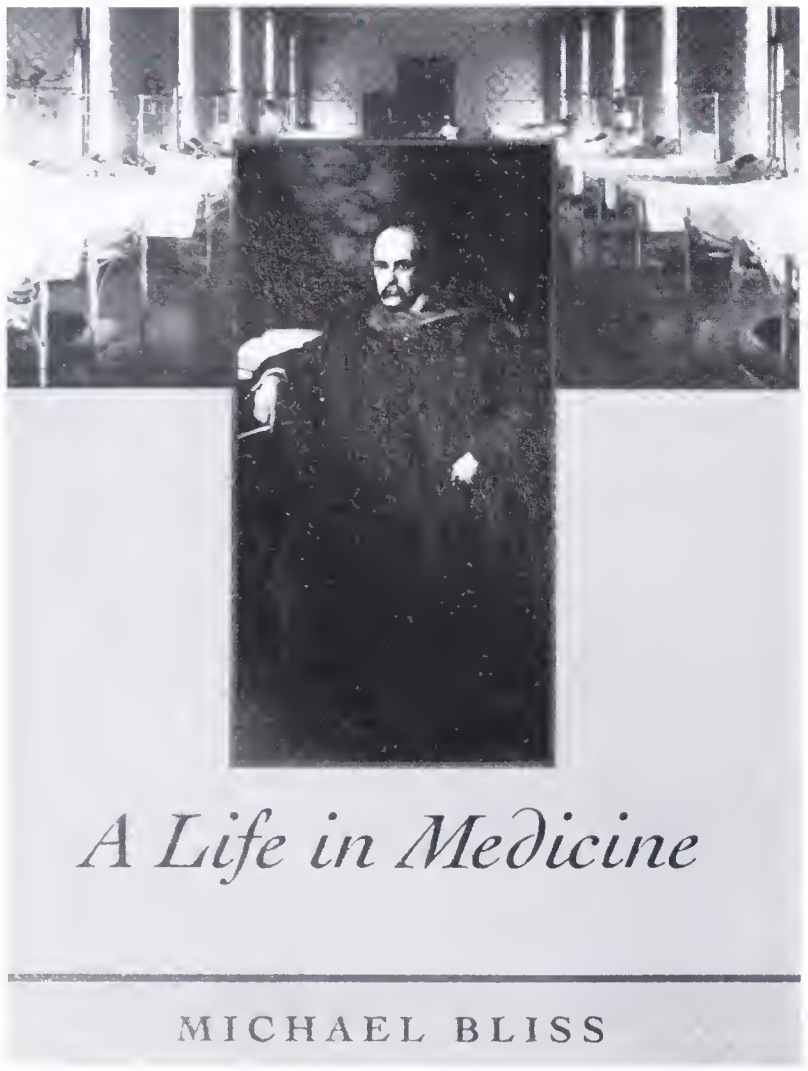
UTMJ Reviews – History of Medicine

William Osler: A Life in Medicine

Reviewed by Irfan Dhalla, B.A.Sc. (OT3)

Written by: Written by Michael Bliss
Publisher: University of Toronto Press
Date of Publication: 1999
Number of pages: 581
Cost: Hardcover edition, $50 (CDN)



HippocPerhaps Aeseulapius, the Greek god of healing, blessed the water Featherstone Osler used to christen his eighth ehild, William. The same morning, in a small town 40 miles north of Toronto, the hardworking priest also baptised Sir Frederiek Banting's father. Sir Frederiek eo-diseovered insulin, and is a household name in Canada. William Osler also excelled: at his death in 1919 he was probably the most beloved physician in the world. Perhaps unsurprisingly, the lay publie has forgotten Osler. What is remarkable, however, is that even within medieine few can reeall much more than his name.

Until now, those who wanted to learn about Osler turned to Harvey Cushing's 1925 Pulitzer prize-winning biography,[1] a 1400-page work of idolatry in dire need of a modern replaeement. Happily, Miehael Bliss, a University of Toronto historian who wrote books about Banting[2] and the diseovery of insulin,[3] dared to take on the ehallenge.

As Bliss tells us, Osler was an intelligent but unexeeptional child. He eame of age in the 1860s, a deeade remembered for its intelleetual elashes between Darwinists and biblical doctrinaires. The young Osler,

who sided with the Darwinists, snubbed the priesthood and chose to study medicine. He graduated from McGill, a medical school where none of the professors used a microscope (they were only just coming into fashion in Europe), and the surgeons still operated without the new aseptic techniques. Still, McGill was one of the best medical schools in North America. Osler graduated third in his class and promptly left for Europe. In those days, though, the post-graduation European jaunt was strictly educational: it was there that the cream of the medical profession practised, and Osler wanted to learn from the best.

Upon his return to Canada at the tender age of 25, Osler was given a faculty position at McGill. His first act was to introduce microscopic histology to the students. Osler quickly built a reputation, first on the basis of his work in pathology and then for his diagnostic acumen. Alas, Canada was too small for him, and after a brief stint at the University of Pennsylvania (where he was chided for his use of the Canadianism "eh"), Osler became the first chief of staff at Johns Hopkins.

At Johns Hopkins, Osler treated everyone from Washington politicians to Baltimore's destitute. He continued to publish case reports and review articles at a furious rate, but teaching remained his greatest joy. Johns Hopkins students met him in their third year, for a novel experience called the "clinical clerkship". Osler loved his students: each Saturday he invited two for dinner and the rest for beer and tobacco. The affection was mutual. An intern at Hopkins wrote, "We all worship him and if it would give Dr. Osler any pleasure to walk over me, I would lie on the ground and let him do it."

Osler retired to become Regius Professor of Medicine at Oxford in 1905. The Oxford sinecure provided him with a decent income, fewer responsibilities, and a more British environment for the Oslers' only child, a son named Revere, whom Osler did not want raised as an American. Ironically, Revere's subsequent love of Britain contributed to his premature death. Against his father's wish, Revere fought and was killed in World War I. Not very long after, at the age of seventy, Osler succumbed to complications from a chest infection.

In telling the story of a life worth celebrating, Bliss's admiration for Osler is displayed on every page, but one cannot fault the author for this. Readers of his Banting biography2 will know that Bliss is fair, and he seems to have investigated every scandalous rumour surrounding Osler only to have found them false. Bliss's prose is crisp, and his book is well researched and referenced. Noticeably absent are developed characterizations of Osler's formidable wife and siblings, but this is a minor quibble.

So why should William Osler be remembered? Osler did not invent any lasting cures or develop any vaccines, though he did practice and teach at the scientific frontier. A colleague once found Osler retching in his office: he was attempting to pass a tube down his own esophagus to see how his patients felt. No wonder his patients thought he was the ideal physician; his peers were equally admiring. Banting, whose greatness rests on one accomplishment, will always be remembered. Osler's greatness cannot be so simply reduced. Nevertheless, readers of Bliss's commendable biography will never forget him.

References

1. Cushing, Harvey. *The Life of Sir William Osler*. Oxford: Clarendon Press. 1925.
2. Bliss, Michael. Banting: *A Biography*. Toronto: McClelland and Stewart. 1984.
3. Bliss, Michael. *The Discovery of Insulin*. Toronto: McClelland and Stewart. 1982.

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2. Kocsis JH, Davis JM, Katz MM, *et al.* (1985). Depressive behavioral and hyperactive adrenocortical function. *Am J Psychiatry*. 142: 1291-1298.

* Abbreviated journal titles should be used.

Articles **with more than 4 authors should include the first three authors followed by *et al.*

Article in a book or comparable publication

3. Smith MHD and Teele DW. (1995). Tuberculosis. *In: Infectious diseases of the fetus and newborn infant, 4th ed.* Remington JS and Klein JO (eds). WB Saunders: Philadelphia, pp. 1074-1086.

4. Podolsky DK and KJ Isselbacher. (1998). Major complications of cirrhosis (ascites). *In: Harrison's Principals of Internal Medicine, 14th ed.* Fauci AS et al. (eds). McGraw-Hill, New York, pp. 210-214.

Books, theses, or comparable publication

5. Pressaco J and Yu H. (1996). *Pharmacological Treatment of Diseases.* Urban Angel Medical Books Inc: Toronto.

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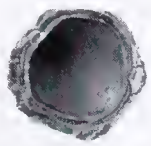

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10 mg, 20 mg and 40 mg tablets

THERAPEUTIC CLASSIFICATION: Lipid Metabolism Regulator

ACTIONS AND CLINICAL PHARMACOLOGY

LIPITOR (atorvastatin calcium) is a synthetic lipid-lowering agent. It is a selective, competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol.

LIPITOR lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic Low Density Lipoprotein (LDL) receptors on the cell-surface for enhanced uptake and catabolism of Low Density Lipoprotein (LDL).

LIPITOR reduces LDL-Cholesterol (LDL-C) and the number of LDL particles. LIPITOR also reduces Very Low Density Lipoprotein-Cholesterol (VLDL-C), serum triglycerides (TG) and Intermediate Density Lipoproteins (IDL), as well as the number of apolipoprotein B (apo B) containing particles, but increases High Density Lipoprotein-Cholesterol (HDL-C). Elevated serum cholesterol due to elevated LDL-C is a major risk factor for the development of cardiovascular disease. Elevated plasma TG is also a risk factor for cardiovascular disease, particularly if due to increased IDL, or associated with decreased HDL-C or increased LDL-C.

Atorvastatin is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours. Atorvastatin tablets are 95% to 99% bioavailable compared to solutions.

Mean distribution of atorvastatin is approximately 381 litres. Atorvastatin is ≥98% bound to plasma proteins. Atorvastatin is extensively metabolized by cytochrome P-450 3A4 to ortho- and para-hydroxylated derivatives and to various beta-oxidation products. Approximately 70% of circulating inhibitory activity for HMG Co-A reductase is attributed to active metabolites.

Atorvastatin and its metabolites are eliminated by biliary excretion. Less than 2% of a dose of atorvastatin is recovered in urine following oral administration. Mean plasma elimination half-life of atorvastatin in humans is approximately 14 hours, but the half-life of inhibitory activity for HMG-CoA reductase is 20 to 30 hours due to the contribution of longer-lived active metabolites.

INDICATIONS AND CLINICAL USE

LIPITOR (atorvastatin calcium) is indicated as an adjunct to diet, at least equivalent to the American Heart Association (AHA) Step 1 diet, for the reduction of elevated total cholesterol, (total-C), LDL-C, TG and apolipoprotein B (apo B) in hyperlipidemic and dyslipidemic conditions, when response to diet and other nonpharmacological measures alone has been inadequate, including:

- Primary hypercholesterolemia (Type IIa),
- Combined (mixed) hyperlipidemia (Type IIb), including familial combined hyperlipidemia, regardless of whether cholesterol or triglycerides are the lipid abnormality of concern;
- Dysbetalipoproteinemia (Type III);
- Hypertriglyceridemia (Type IV);
- Familial hypercholesterolemia (homozygous and heterozygous). For homozygous familial hypercholesterolemia, LIPITOR should be used as an adjunct to treatments such as LDL apheresis, or as monotherapy if such treatments are not available.

In clinical trials, LIPITOR (10 to 80 mg/day) significantly improved lipid profiles in patients with a wide variety of hyperlipidemic and dyslipidemic conditions. In 2 dose-response studies in mildly-to-moderately hyperlipidemic patients (Fredrickson Types IIa and IIb), LIPITOR reduced the levels of total cholesterol (29-45%), LDL-C (39-60%), apo B (32-50%), TG (19-37%), and increased high density lipoprotein cholesterol (HDL-C) levels (5-9%). Comparable responses were achieved in patients with heterozygous familial hypercholesterolemia, non-familial forms of hypercholesterolemia, combined hyperlipidemia, including familial combined hyperlipidemia and patients with non-insulin dependent diabetes mellitus. In patients with hypertriglyceridemia (Type IV), LIPITOR (10 to 80 mg daily) reduced TG (25 - 56%) and LDL-C levels (23 - 40%). Chylomicrons, which characterize Types I and V, have not been measured in clinical studies in patients with high TG levels (>11 mmol/L).

In an open-label study in patients with dysbetalipoproteinemia (Type III), LIPITOR (10 to 80 mg daily) reduced total-C (40-57%), TG (40-56%) and IDL-C + VLDL-C levels (34-58%).

In an open label study in patients with homozygous familial hypercholesterolemia (FH) LIPITOR (10 to 80 mg daily) reduced mean LDL-C levels (22%). In a pilot study, LIPITOR 80 mg/day showed a mean LDL-C lowering of 30% for patients not on plasmapheresis and of 31% for patients who continued plasmapheresis. A mean LDL-C lowering of 35% was observed in receptor defective patients and of 19% in receptor negative patients (see PHARMACOLOGY, Clinical Studies).

For more details on efficacy results by pre-defined classification and pooled data by Fredrickson types, see PHARMACOLOGY, Clinical Studies.

Prior to initiating therapy with LIPITOR, secondary causes should be excluded for elevations in plasma lipid levels (e.g. poorly controlled diabetes mellitus, hypothyroidism, nephrotic syndrome, dysproteinemias, obstructive liver disease, and alcoholism), and a lipid profile performed to measure total cholesterol, LDL-C, HDL-C, and TG. For patients with TG <4.52 mmol/L (<400 mg/dL), LDL-C can be estimated using the following equation:

$$\text{LDL-C (mmol/L)} = \text{total-C} - [(0.37 \times \text{(TG)}) + \text{HDL-C})]$$

$$\text{LDL-C (mg/dL)} = \text{total-C} - [(0.2 \times \text{(TG)}) + \text{HDL-C})]^{1}$$

For patients with TG levels >4.52 mmol/L (>400 mg/dL), this equation is less accurate and LDL-C concentrations should be measured directly or by ultracentrifugation.

CONTRAINDICATIONS

Hypersensitivity to any component of this medication.

Active liver disease or unexplained persistent elevations of serum transaminases exceeding 3 times the upper limit of normal (see WARNINGS).

Pregnancy and lactation (see PRECAUTIONS).

WARNINGS

Pharmacokinetic Interactions

The use of HMG-CoA reductase inhibitors has been associated with severe myopathy, including rhabdomyolysis, which may be more frequent when they are co-administered with drugs that inhibit the cytochrome P-450 enzyme system. Atorvastatin is metabolized by cytochrome P-450 isoform 3A4 and as such may interact with agents that inhibit this enzyme. (See WARNINGS, Muscle effects and PRECAUTIONS, Drug Interactions and Cytochrome P-450-mediated Interactions).

Hepatic Effects

In clinical trials, persistent increases in serum transaminases greater than three times the upper limit of normal occurred in <1% of patients who received LIPITOR. When the dosage of LIPITOR was reduced, or when drug treatment was interrupted or discontinued, serum transaminase levels returned to pretreatment levels. The increases were generally not associated with jaundice or other clinical signs or symptoms. Most patients continued treatment with a reduced dose of LIPITOR without clinical sequelae.

Liver function tests should be performed before the initiation of treatment, and periodically thereafter. Special attention should be paid to patients who develop elevated serum transaminase levels, and in these patients measurements should be repeated promptly and then performed more frequently

If increases in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) show evidence of progression, particularly if they rise to greater than 3 times the upper limit of normal and are persistent, the dosage should be reduced or the drug discontinued.

LIPITOR should be used with caution in patients who consume substantial quantities of alcohol and/or have a past history of liver disease. Active liver disease or unexplained transaminase elevations are contraindications to the use of LIPITOR; if such a condition should develop during therapy, the drug should be discontinued.

Muscle Effects

Myopathy, defined as muscle aching or muscle weakness in conjunction with increases in creatine phosphokinase (CPK) values to greater than ten times the upper limit of normal, should be considered in any patient with diffuse myalgia, muscle tenderness or weakness, and/or marked elevation of CPK. Patients should be advised to report promptly unexplained muscle pain, tenderness or weakness, particularly if accompanied by malaise or fever. LIPITOR therapy should be discontinued if markedly elevated CPK levels occur or myopathy is diagnosed or suspected.

The risk of myopathy and rhabdomyolysis during treatment with HMG-CoA reductase inhibitors is increased with concurrent administration of cyclosporin, fibric acid derivatives, erythromycin, niacin (nicotinic acid), azole antifungals or nefazodone. As there is no experience to date with the use of LIPITOR given concurrently with these drugs, with the exception of a pharmacokinetic study with erythromycin, the benefits and risks of such combined therapy should be carefully considered (see PRECAUTIONS, Drug Interactions)

Rhabdomyolysis has been reported in very rare cases with LIPITOR (see PRECAUTIONS, Drug Interactions).

Rhabdomyolysis with renal dysfunction secondary to myoglobinuria has also been reported with HMG-CoA reductase inhibitors.

LIPITOR therapy should be temporarily withheld or discontinued in any patient with an acute serious condition suggestive of a myopathy or having a risk factor predisposing to the development of renal failure secondary to rhabdomyolysis (such as severe acute infection, hypotension, major surgery, trauma, severe metabolic, endocrine and electrolyte disorders, and uncontrolled seizures).

PRECAUTIONS

General

The effects of atorvastatin-induced changes in lipoprotein levels, including reduction of serum cholesterol on cardiovascular morbidity or mortality or total mortality have not been established.

Before instituting therapy with LIPITOR (atorvastatin calcium), an attempt should be made to control elevated serum lipoprotein levels with appropriate diet, exercise, and weight reduction in overweight patients, and to treat other underlying medical problems (see INDICATIONS AND CLINICAL USE). Patients should be advised to inform subsequent physicians of the prior use of LIPITOR or any other lipid-lowering agents.

Effect on the Lens

Current long-term data from clinical trials do not indicate an adverse effect of atorvastatin on the human lens.

Effect on Ubiquinone (CoQ_{10}) Levels

Significant decreases in circulating ubiquinone levels in patients treated with atorvastatin and other statins have been observed. The clinical significance of a potential long-term statin-induced deficiency of ubiquinone has not been established. It has been reported that a decrease in myocardial ubiquinone levels could lead to impaired cardiac function in patents with borderline congestive heart failure (see SELECTED BIBLIOGRAPHY)

Effect on Lipoprotein (a)

In some patients, the beneficial effect of lowered total cholesterol and LDL-C levels may be partly blunted by a concomitant increase in Lp(a) levels. Until further experience is obtained, it is suggested, where feasible, that measurements of serum Lp(a) be followed up in patients placed on atorvastatin therapy (see SELECTED BIBLIOGRAPHY).

Hypersensitivity

An apparent hypersensitivity syndrome has been reported with other HMG-CoA reductase inhibitors which has included 1 or more of the following features: anaphylaxis, angioedema, lupus erythematous-like syndrome, polymyalgia rheumatica, vasculitis, purpura, thrombocytopenia, leukopenia, hemolytic anemia, positive ANA, ESR increase, eosinophilia, arthritis, arthralgia, urticaria, asthenia, photosensitivity, fever, chills, flushing, malaise, dyspnea, toxic epidermal necrolysis, erythema multiforme, including Stevens-Johnson syndrome. Although to date hypersensitivity syndrome has not been described as such, LIPITOR should be discontinued if hypersensitivity is suspected

Use in Pregnancy

LIPITOR is contraindicated during pregnancy (see CONTRAINDICATIONS).

Atherosclerosis is a chronic process and discontinuation of lipid-lowering drugs during pregnancy should have little impact on the outcome of long-term therapy of primary hypercholesterolemia. Cholesterol and other products of cholesterol biosynthesis are essential components for fetal development (including synthesis of steroids and cell membranes). Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other biologically active substances derived from cholesterol, they may cause harm to the fetus when administered to pregnant women.

There are no data on the use of LIPITOR during pregnancy. LIPITOR should be administered to women of childbearing age only when such patients are highly unlikely to conceive and have been informed of the potential hazards. If the patient becomes pregnant while taking LIPITOR, the drug should be discontinued and the patient apprised of the potential risk to the fetus.

Nursing Mothers

In rats, milk concentrations of atorvastatin are similar to those in plasma. It is not known whether this drug is excreted in human milk. Because of the potential for adverse reactions in nursing infants, women taking LIPITOR should not breast-feed (see CONTRAINDICATIONS).

Pediatric Use

Treatment experience in a pediatric population is limited to doses of LIPITOR up to 80 mg/day for 1 year in 8 patients with homozygous familial hypercholesterolemia. No clinical or biochemical abnormalities were reported in these patients.

Geriatric Use

Treatment experience in adults 70 years or older (N=221) with doses of LIPITOR up to 80 mg/day has demonstrated that the safety and effectiveness of atorvastatin in this population was similar to that of patients <70 years of age. Pharmacokinetic evaluation of atorvastatin in subjects over the age of 65 years indicates an increased AUC. As a precautionary measure, the lowest dose should be administered initially (see PHARMACOLOGY, Human Pharmacokinetics; SELECTED BIBLIOGRAPHY)

Renal Insufficiency

Plasma concentrations and LDL-C lowering efficacy of LIPITOR was shown to be similar in patients with moderate renal insufficiency compared with patients with normal renal function. However, since several cases of rhabdomyolysis have been reported in patients with a history of renal insufficiency of unknown severity, as a precautionary measure and pending further experience in renal disease, the lowest dose (10 mg/day) of LIPITOR should be used in these patients. Similar precautions apply in patients with severe renal insufficiency [creatinine clearance <30 mL/min (<0.5 mL/sec)]; the lowest dosage should be used and implemented cautiously (see WARNINGS, Muscle Effects, PRECAUTIONS, Drug Interactions).

Refer also to DOSAGE AND ADMINISTRATION

Endocrine Function

HMG-CoA reductase inhibitors interfere with cholesterol synthesis and as such might theoretically blunt adrenal and/or gonadal steroid production. Clinical studies with atorvastatin and other HMG-CoA reductase inhibitors have suggested that these agents do not reduce plasma cortisol concentration or impair adrenal reserve and do not reduce basal plasma testosterone concentration. However, the effects of HMG-CoA reductase inhibitors on male fertility have not been studied in adequate numbers of patients. The effects, if any, on the pituitary gonadal axis in premenopausal women are unknown.

Patients treated with atorvastatin who develop clinical evidence of endocrine dysfunction should be evaluated appropriately. Caution should be exercised if an HMG-CoA reductase inhibitor or other agent used to lower cholesterol levels is administered to patients receiving other drugs (e.g. ketoconazole, spironolactone or cimetidine) that may decrease the levels of endogenous steroid hormones.

Drug Interactions

Concomitant Therapy with Other Lipid Metabolism Regulators: Combined drug therapy should be approached with caution as information from controlled studies is limited.

[1]Friedewald, WT et al. *Clin Chem* 1972; 18(6): 489-502

...Acid Sequestrants:

...greater when LIPITOR 10 mg and colestipol ... when [illegible] was administered alone [35% for LIPITOR and 22% for ...

...cholesterol reduction was similar (53%) when LIPITOR 40 mg and colestipol 20 g ... when compared to that with LIPITOR 80 mg alone. Plasma concentration of atorvastatin was lower ... when LIPITOR 40 mg plus colestipol 20 g were coadministered compared with LIPITOR 40 mg ...

...therapy was less effective in lowering the triglycerides than LIPITOR monotherapy in ... patients (see PHARMACOLOGY, Clinical Studies).

... with colestipol or any other resin, an interval of at least 2 hours should be ... between the two drugs, since the absorption of LIPITOR may be impaired by the resin.

Fibric Acid Derivatives (Gemfibrozil, Fenofibrate, Bezafibrate) and Niacin (Nicotinic Acid): Although there is ... with the use of LIPITOR given concurrently with fibric acid derivatives and niacin, the benefits and risks ... therapy should be carefully considered. The risk of myopathy during treatment with other drugs in ... increased with concurrent administration (see WARNINGS, Muscle Effects).

Coumarin Anticoagulants: LIPITOR had no clinically significant effect on prothrombin time when administered to patients receiving long-term warfarin therapy (see SELECTED BIBLIOGRAPHY).

Digoxin: Coadministration of multiple doses of LIPITOR and digoxin increased steady-state plasma digoxin concentrations by approximately 20%. Patients taking digoxin should be monitored closely and appropriately.

Oral Contraceptives: Coadministration of LIPITOR with an oral contraceptive, containing 1mg norethindrone and 35µg ethinyl estradiol, increased plasma concentrations (AUC levels) of norethindrone and ethinyl estradiol by approximately ...% and 2...%, respectively. These increases should be considered when selecting an oral contraceptive.

Antacids: Administration of aluminum and magnesium based antacids, such as Maalox® TC Suspension, with LIPITOR decreased plasma concentrations of LIPITOR by approximately 35%. LDL-C reduction was not altered but the triglyceride lowering effect of LIPITOR may be affected.

Cimetidine: Administration of cimetidine with LIPITOR did not alter plasma concentrations or LDL-C lowering efficacy of LIPITOR; however, the triglyceride lowering effect of LIPITOR was reduced from 34% to 26%.

Cytochrome P-450-mediated Interactions: Atorvastatin is metabolized by the cytochrome P-450 isoenzyme, CYP 3A4. Erythromycin, a CYP 3A4 inhibitor, increased atorvastatin plasma levels by 40%. Coadministration of CYP 3A4 inhibitors such as grapefruit juice, macrolide antibiotics (including erythromycin and clarithromycin), immunosuppressants (cyclosporine), azole antifungal agents (i.e. itraconazole, ketoconazole), or the antidepressant nefazodone may have the potential to increase plasma concentrations of HMG-CoA reductase inhibitors, including LIPITOR (see SELECTED BIBLIOGRAPHY). Caution should thus be exercised with concomitant use of these agents (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Renal Insufficiency and Endocrine Function; DOSAGE AND ADMINISTRATION; SELECTED BIBLIOGRAPHY).

In a study with healthy subjects, coadministration of maximum doses of both atorvastatin (80 mg) and terfenadine (120 mg), a CYP 3A4 substrate, was shown to produce a modest increase in terfenadine AUC. The QTc interval remained unchanged. However, since an interaction between these two drugs cannot be excluded in patients with predisposing factors for arrhythmia, (e.g. preexisting prolonged QT interval, severe coronary artery disease, hypokalemia), caution should be exercised when these agents are coadministered (see WARNINGS, Pharmacokinetic Interactions; DOSAGE AND ADMINISTRATION).

Antipyrine: Antipyrine was used as a non-specific model for drugs metabolized by the microsomal hepatic enzyme system (cytochrome P-450 system). LIPITOR had no effect on the pharmacokinetics of antipyrine, thus interactions with other drugs metabolized via the same cytochrome isozymes are not expected.

Erythromycin: In healthy individuals, plasma concentrations of atorvastatin increased approximately 40% with coadministration of LIPITOR and erythromycin, a known inhibitor of CYP 3A4 (see WARNINGS, Muscle Effects).

Other Concomitant Therapy: In clinical studies, LIPITOR was used concomitantly with antihypertensive agents and estrogen replacement therapy without evidence to date of clinically significant adverse interactions. Interaction studies with specific agents have not been conducted.

Patients with Severe Hypercholesterolemia: Higher drug dosages (80 mg/day) required for some patients with severe hypercholesterolemia (including familial hypercholesterolemia) are associated with increased plasma levels of atorvastatin. **Caution should be exercised in such patients who are also severely renally impaired, elderly, or are concomitantly being administered digoxin or CYP 3A4 inhibitors (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Drug Interactions; DOSAGE AND ADMINISTRATION).**

Drug/Laboratory Test Interactions

LIPITOR may elevate serum transaminase and creatine phosphokinase levels (from skeletal muscle). In the differential diagnosis of chest pain in a patient on therapy with LIPITOR, cardiac and noncardiac fractions of these enzymes should be determined.

ADVERSE REACTIONS

LIPITOR is generally well tolerated. Adverse reactions have usually been mild and transient. In controlled clinical studies (placebo-controlled and active-controlled comparative studies with other lipid-lowering agents) involving 2502 patients, <2% of patients were discontinued due to adverse experiences attributable to LIPITOR. Of these 2502 patients, 1721 were treated for at least 6 months and 1253 for 1 year or more.

Adverse experiences occurring at an incidence ≥1% in patients participating in placebo-controlled clinical studies of LIPITOR and reported to be possibly, probably or definitely drug related are shown in Table 1 below.

TABLE 1. Associated Adverse Events Reported in ≥1% of Patients in Placebo Controlled Clinical Trials

	Placebo % (n=270)	LIPITOR % (n=1122)
GASTROINTESTINAL		
Constipation	1	1
Diarrhea	1	1
Dyspepsia	2	1
Flatulence	2	1
Nausea	0	1
NERVOUS SYSTEM		
Headache	2	1
MISCELLANEOUS		
Pain	<1	1
Myalgia	1	1
Asthenia	<1	1

The following additional adverse events were reported in clinical trials, not all events listed below have been associated with a causal relationship to LIPITOR therapy. Muscle cramps, myositis, myopathy, paresthesia, peripheral neuropathy, pancreatitis, hepatitic cholestatic jaundice, anorexia, vomiting, alopecia, pruritus, rash, impotence, hyperglycemia, and hypoglycemia.

Post-marketing experience: Very rare reports of severe myopathy with or without rhabdomyolysis have been reported (see WARNINGS, Muscle Effects; PRECAUTIONS, Renal Insufficiency and Drug Interactions). Isolated cases of thrombocytopenia and allergic reactions (including urticaria, angioneurotic edema and anaphylaxis) that may have no causal relationship to atorvastatin have also been reported.

...(laboratory observations, see PRECAUTIONS).

...(laboratory Tests): Increases in serum transaminase levels have been noted in clinical trials (see WARNINGS).

SYMPTOMS AND TREATMENT OF OVERDOSAGE

...specific treatment for atorvastatin overdosage. Should an overdose occur, the patient should be treated ... and supportive measures instituted as required. Due to extensive drug binding to plasma proteins, ... not expected to significantly enhance atorvastatin clearance.

DOSAGE AND ADMINISTRATION

... cholesterol lowering diet (at least equivalent to the American Heart Association ... before beginning therapy with LIPITOR, and should continue on this diet during treatment with LIPITOR. If ... physical exercise should be implemented.

Primary Hypercholesterolemia and Combined (Mixed) Hyperlipidemia, Including Familial Combined Hyperlipidemia

The recommended dose of LIPITOR is 10 mg once a day. The majority of patients achieve and maintain target cholesterol levels with LIPITOR 10 mg/day. A significant therapeutic response is evident within 2 weeks, and the maximum response is usually achieved within 2-4 weeks. The response is maintained during chronic therapy.

Doses can be given at any time of the day, with or without food, and should preferably be given in the evening. Doses should be individualized according to baseline LDL-C and/or TG levels, the desired LDL-C and/or TG target (see the Detection and Management of Hypercholesterolemia, Working Group on Hypercholesterolemia and other Dyslipidemias [Canada] and/or the US National Cholesterol Education Program [NCEP]), the goal of therapy and the patient's response. Adjustments of dosage, if necessary, should be made at intervals of 4 weeks or more. The recommended dose range for most patients is 10 to 40 mg/day. The maximum dose is 80 mg/day, which may be required in a minority of patients (see section below).

Lipid levels should be monitored periodically and, if necessary, the dose of LIPITOR adjusted based on target lipid levels recommended by guidelines.

The following reductions in total cholesterol and LDL-C levels have been observed in 2 dose-response studies, and may serve as a guide to treatment of patients with mild to moderate hypercholesterolemia.

TABLE 2. Dose-Response in Patients With Mild to Moderate Hypercholesterolemia
(Mean Percent Change from Baseline)*

Lipid Parameter	LIPITOR Dose (mg/day)			
	10 (N=22)	20 (N=20)	40 (N=21)	80 (N=23)
Total-C: 7.1 mmol/L† (273 mg/dL)†	-29	-33	-37	-45
LDL-C: 4.9 mmol/L† (190 mg/dL)†	-39	-43	-50	-60

*Results are pooled from 2 dose-response studies.
†Mean baseline values.

Severe Dystipidemias:

In patients with severe dyslipidemias, including homozygous and heterozygous familial hypercholesterolemia and dysbetalipoproteinemia (Type III), higher dosages (up to 80 mg/day) may be required (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Drug Interactions).

Concomitant Therapy

See PRECAUTIONS, Drug Interactions.

Dosage in Patients With Renal Insufficiency

See PRECAUTIONS.

PHARMACEUTICAL INFORMATION

Drug Substance

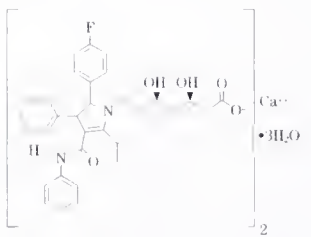
Proper Name: Atorvastatin calcium

Chemical Name: [R-(R*,R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)-carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate

Empirical Formula: $(C_{33}H_{34}FN_2O_5)_2Ca \cdot 3H_2O$

Molecular Weight: 1209.42

Structural Formula:



Description: Atorvastatin calcium is a white to off-white crystalline powder that is practically insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

Tablet Composition:

Each tablet contains either 10 mg, 20 mg or 40 mg atorvastatin as the active ingredient. Each tablet also contains the following non-medicinal ingredients: calcium carbonate, candelilla wax, croscarmellose sodium, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, hydroxypropyl methylcellulose, polyethylene glycol, talc, titanium dioxide, polysorbate 80 and simethicone emulsion.

Stability and Storage Recommendations:

Store at controlled room temperature 15 to 25°C.

AVAILABILITY OF DOSAGE FORMS

LIPITOR (atorvastatin calcium) is available in dosage strengths of 10 mg, 20 mg and 40 mg atorvastatin per tablet.

10 mg: White, elliptical, film-coated tablet, coded "10" on one side and "PD 155" on the other. Available in bottles of 90 tablets.

20 mg: White, elliptical, film-coated tablet, coded "20" on one side and "PD 156" on the other. Available in bottles of 90 tablets.

40 mg: White, elliptical, film-coated tablet, coded "40" on one side and "PD 157" on the other. Available in bottles of 90 tablets.

References:

1. Koren MJ, Smith DG, Hunninghake DB, *et al*. The cost of reaching National Cholesterol Education Program goals in hypercholesterolemic patients: A comparison of atorvastatin, simvastatin, lovastatin and fluvastatin. *Pharmacoeconomics* 1998;14:59-70. 2. LIPITOR (atorvastatin calcium) Product Monograph, Parke-Davis Div., Warner-Lambert Canada Inc., Dec. 1998. 3. Dart A, *et al*. A multicenter, double-blind, one-year study comparing safety and efficacy of atorvastatin versus simvastatin in patients with hypercholesterolemia. *Am J Cardiol* 1997;80:39-44. 4. Bertolini S, *et al*. Efficacy and safety of atorvastatin compared to pravastatin in patients with hypercholesterolemia. *Atherosclerosis* 1997;130:191-7. 5. Data on file. 6. ODB Formulary, Dec. 1998.

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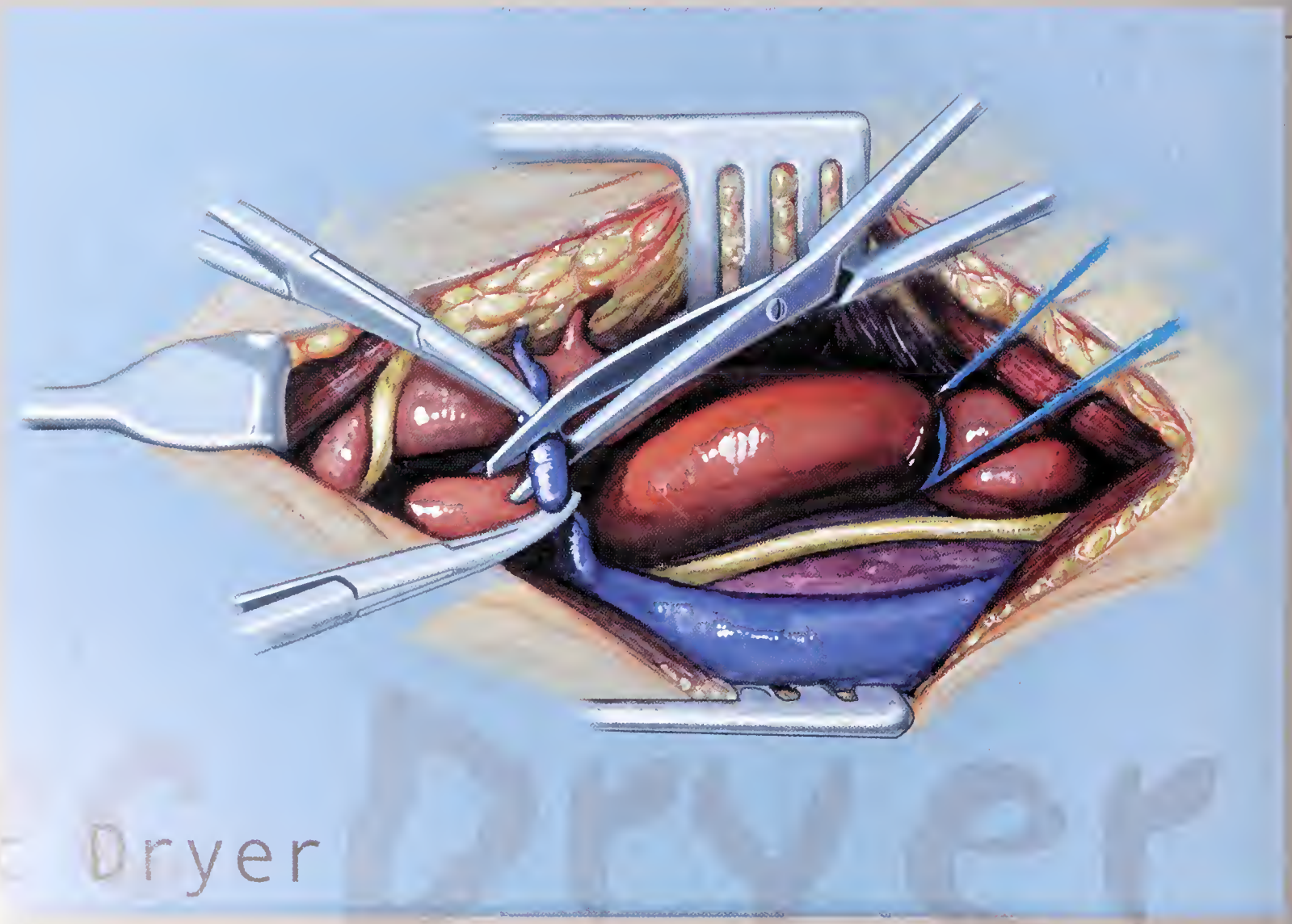

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